Chapter 2

Lipid Oxidation in Homogeneous and Micro-Heterogeneous Media in Presence of Prooxidants, Antioxidants and Surfactants

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Additional information is available at the end of the chapter

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1. Introduction

The human body is constantly subjected to a significant oxidative stress as a result of the misbalance between antioxidative protective systems and the formation of strong oxidizing substances, including free radicals. The stress can damage DNA, proteins, lipids and carbohydrates and could cause negative effect to intracellular signal transmission. Antioxidants could be promising agents for management of oxidative stress-related diseases. Oxygen is essential for all living organisms, but at the same time it is a source of constant aggression for them. In its ground triplet state ($^3O_2$) oxygen has weak reactivity, but it can produce strongly aggressive and reactive particles such as singlet state oxygen ($^1O_2$), hydroperoxides (H_2O_2), superoxide anion ($O_2^{\bullet -}$), hydroxylic radical ($OH^{\bullet}$) and various peroxide ($LO_2^{\bullet}$) and alkoxy radicals ($LO^{\bullet}$). It is well known that the latter lead to an oxidative degradation of biological macromolecules, changing their properties and thus the cell structure and functionality. The free radicals formation in the hydrophobic parts of the biological membranes initiates radical disintegration of the hydrocarbon "tails" of the lipids. This process is known as lipid peroxidation (Figure 1) [1-3].

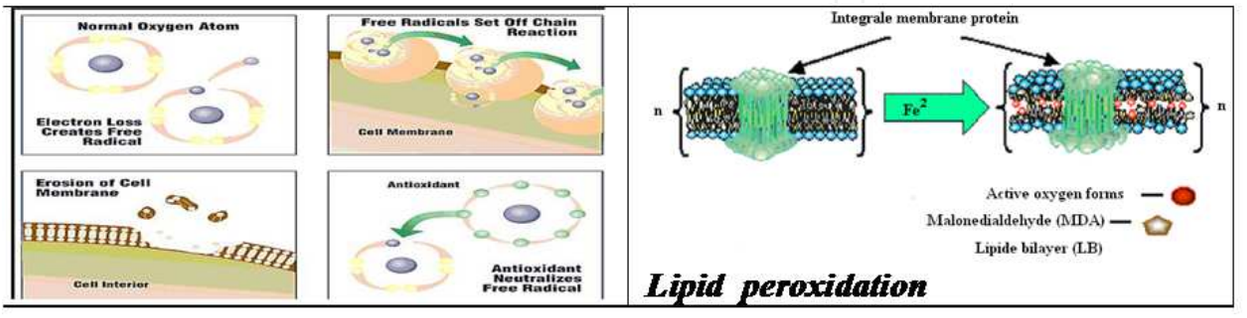


Figure 1. Erosion of cell membrane, antioxidant neutralizes free radicals and lipid peroxidation

In recent decades, many communications have been devoted to the significant role of physical factors, which control the structure of nutrition systems, in the chemistry of lipid oxidation in oil/water (O/W) emulsions [4,5]. In this case, the rate of lipid oxidation strongly depends on the physical properties of interfaces, because they affect the character of the interaction between water-soluble compounds of transition metals and hydroperoxides located inside and on the surface of emulsion droplets. For example, positively charged and high viscosity interfaces that hinder the contact between iron ions and hydroperoxides inhibit oxidation of fat emulsions [6,7]. Another example of the influence of physical factors on the oxidation is the antioxidant polar paradox [6-9], which is based on the fact that nonpolar antioxidants are efficient in O/W emulsions, because they are located in emulsion droplets together with oxidizable lipids. Polar antioxidants are more efficient in W/O emulsions because they are concentrated at the interfaces [10-12]. In the frame of this chapter, the main features of lipid oxidation in homogeneous and micro - heterogeneous oil media, formed by surfactants (W/O microemulsions) will be discussed.

2. Kinetic model of homogeneous lipid oxidation

The kinetic model is based (Table 1) on the reactions and corresponding rate constants known for the oxidation of methyl linoleate (MeLi), because linoleic esters are easily oxidizable components of many natural lipid systems and thus determines the oxidizability of the lipid substrates.

The kinetic scheme of liquid-phase (homogeneous) oxidation of lipids (LH) includes reactions 1-12.

In the presence of an initiator (I), i.e. initiated oxidation, the formation of radicals occurs with a constant initiation rate $R_{IN}=2k_1[I]$. Under autoxidation conditions ([I]=0), the rates of radical formation in the reactions of LH with O_2 (reaction 6) and decomposition of lipid hydroperoxides (LOOH) (reactions 7 and 8) increase as LOOH is accumulated. The chain termination occurs due to recombination or disproportionation of radicals (reactions 9, 11 and 12). The scheme of inhibited oxidation includes reactions 13-19, known for the phenolic antioxidants, AH.

The calculations was performed for three groups of AH, which differ in their activity in reaction 13 with peroxyl radicals LOO: group I (of the type of alpha-tocopherol) with $k_{13}=1.5\ 10^6 M^{-1}s^{-1}$; group II (AH of type of unhindered phenols, e.g. hydroquinone), with $k_{13}=1.5\ 10^5 M^{-1}s^{-1}$; and group III (AH of the type of sterically hindered phenols, e.g. butylated hydroxyl toluene, BHT with $k_{13}=1.5\ 10^4 M^{-1}s^{-1}$. The effect of AH regeneration in reaction 16 was considered for rapidly ($k_{16}=4\ 10^7 M^{-1}s^{-1}$) and slowly ($k_{16}=3\ 10^3 M^{-1}s^{-1}$) reacting phenoxyl radicals A·. Reaction 19 is the chain transfer by the inhibitor radical. It can be a hydrogen abstraction from the substrate molecule with regeneration of the inhibitor. Chain transfer can occur as the addition of A· to unsaturated bonds of polyene compounds, in this case, the inhibitor is not regenerated [13]. We examined both cases, rate constants k_{19} were varied within the 0-100 $M^{-1}s^{-1}$ range (taking into account the published data). In this series of calculations, we accepted that reaction 16 occurs as disproportionation.

No	Reaction	k/M^{-1}s^{-1} , MeLi	Refs.	k/M^{-1}s^{-1}, Limonene
1	I → P + I·	5 10^{-6}	13	5 10^{-6}
2	I· + O_2 → IO_2·	5 10^{6}	13	5 10^{6}
3	IO_2· + LH → LO_2· + IOOH	1 10^{3}	13	1 10^{3}
4	L· + O_2 → LO_2·	5 10^{6} 1 10^{8}	13 15	1.5 10^{7}
5	LO_2· + LH → LO_2· + LOOH	90 100	13 15,16	14
6	LH + O_2 → L· + HO_2·	5.8 10^{-11}	13	1.2 10^{-14}
7	LOOH+LH → L· + LO_2 + H_2O	2.3 10^{-7}	13	4 10^{-8}
8	2LOOH → LO· + LO_2 + H_2O	2.4 10^{-6}	13	1 10^{-6}
9	2 LO_2· → Alc + Ket	1 10^{5} 4.4 10^{6} 1 10^{7}	17 13,18 15	3.5 10^{6}
10	LO· + LH → Alc + Ket	1 10^{7} 1 10^{5}	19 13	1.7 10^{7}
11	LO· + LO_2· → Ket + LOOH	5 10^{6}	13	
12	LO_2· + IO_2· → Alc + Ket	5 10^{6}	13	5 10^{6}
13	AH + LO_2· → A· + LOOH	1.5 10^{4} -1.5 10^{6} 2 10^{6}	13,16 15	
14	AH + LO· → A· + Alc	1 10^{7}	13	
15	AH + IO_2· → A · + IOOH	1.5 10^{5}	13	
16	2A· → P1 (+AH)	3 10^{3} – 4 10^{7}	13,16,18	
17	A· + LO_2· → P2	2.5 10^{6} 3 10^{8}	20 13,16	
18	A· + LO· → P3 +AH + Ket	3 10^{8}	13	
19	A· + LH → L· + (AH/P4)	0-100 0.07	13 21	

Note: The rate constants (k_0 correspond to the oxidation of MeLi at 60°C; in reaction 1, 2 and 4, k are presented in s^{-1}. Initial concentrations: [LH]=2.9M, $[LOOH]_0=10^{-5}$M, [I]=4 10^{-3}M, $[AH]_0=10^{-4}$M, $[O_2]=10^{-3}$M=const; oxidation usually occurs at a constant oxygen pressure, therefore $[O_2]$ is included in the corresponding rate constants: $k_2=k_4=k_6=k_i[O_2]$.

Table 1. The Approximate Rate Constants of the Different Reactions Involved in the Autoxidation of Methyl Linoleate [13] and Limonene [14] in initiated oxidation, autoxidation and inhibited oxidation (at 60°C).

The main kinetic parameters	Initiated oxidation	Lipid autoxidation
Rate of initiation (R_{IN})	Constant and well-controlled $R_{IN}=2k_1[I]$	$R_{IN}=2k_6[LH][O_2]$ + $2k_7[LH][LOOH]+2k_8[LOOH]^2$
Rate of oxidation (R_0) and (R_A)	$R_0=k_p\,[LH](R_{IN}/2k_t)^{0.5}$	
Rate of non-inhibited oxidation (R_0)	$R_A=k_p[LH]R_{IN}/nk_A[AH]_0$	$R_0=k_p\,[LH](R_{IN}/k_t)^{0.5}$
Rate of inhibited oxidation (R_A)	$k_p = k_5$; $k_t = k_9$	$R_A=k_p[LH]R_{IN}/nk_A[AH]_0$
Oxidizability parameter	$a = k_p/(2k_t)^{0.5}$	$a = k_p/(2k_t)^{0.5}$
Inhibition degree (ID)	$ID = \nu_0/\nu_A$	$ID = R_0/R_A$
Induction period (IP)	$IP=n[AH]_0/R_{IN}$	$IP=n[AH]_0/R_{IN}$
Antioxidant efficiency	nk_A	$PF=IP_A/IP_0$ and $RAE=(IP_A-IP_0)/IP_0$

Table 2. The main kinetic parameters of initiated oxidation and lipid autoxidation

Under other equivalent conditions, the bimolecular decay of 2A· by disproportionation in which AH is regenerated gives a considerable advantage in retardation effects as compared with the situation where no regeneration occurs (recombination of 2A·). The presence of the second hydroxyl group in the aromatic ring results in higher k_{16}. In this case, an increase in the induction period related to AH regeneration is most pronounced.

Lipid oxidation is one of the important reactions in biology. Chemical reaction kinetics considers two aspects: the rate of reaction and effective factors – temperature concentration of reactants and products. This knowledge is an essential prerequisite for modeling the lipid oxidation, the shelf life of stored foods, durability of low density proteins, and so on.

3. Effect of pro-oxidants (ROH) leading to acceleration of lipid hydroperoxides (LOOH) decomposition

3.1. Kinetic modeling of lipid oxidation for different mechanism of LOOH decomposition

A kinetic analysis of non-inhibited lipid (LH) autoxidation for different mechanisms of hydroperoxides (LOOH) decay is proposed [22]. It is based on using of mathematical simulation methods of LH autoxidation kinetics. Kinetic schemes of LH autoxidation for some different ways of hydroperoxides decay - mono-molecular, pseudo-mono-molecular and/or bimolecular mechanism are presented. This analysis permits establishing the influence degree of different hydroperoxides decay mechanisms on the kinetic parameters, characterizing the substrate oxidizability. The proposed kinetic analysis has been applied to the methyl linoleate, MeLi) autoxidation at 60°C.

The kinetic model that describes the lipid hydroperoxides decomposition taking into account the possibility of monomolecular (LOOH), pseudo-monomolecular (LOOH + LH) and bimolecular (2 LOOH) mechanisms in both cases: in presence of an oxygen (O_2) and in its absence, i.e. in an inert atmosphere (N_2) is illustrated by Scheme 1. In these equations:

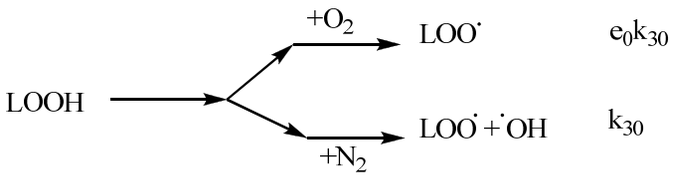
$$[LOOH] = \frac{1 + K_1[LH]}{2K_2}\left[\sqrt{1 + \frac{4K_2[T]}{(1 + K_1[LH]}} - 1\right]$$

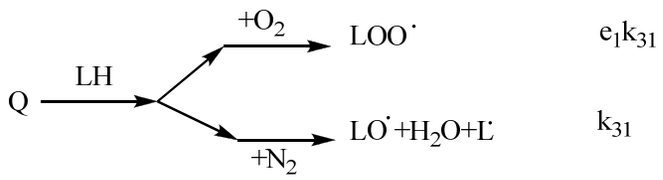
$$[T] = \left(\sqrt{[To]} + \frac{1}{2}\frac{kp}{\sqrt{kt}}[LH]\sqrt{k_i}\, t\right)^2$$

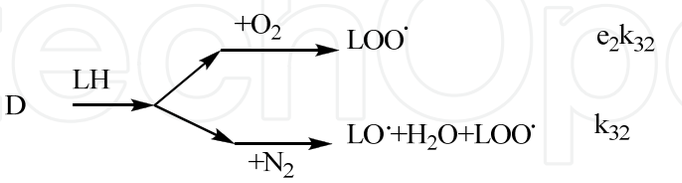
$$C = \frac{4K_2}{(1 + K_1[LH])^2}$$

$$d = \frac{e_o k_{3o} + e_1 k_{31} K_1[LH]}{1 + K_1[LH]} = k_i$$

$$[O_2] = \frac{k_p}{\sqrt{k_t}}[\mathrm{LH}]\sqrt{k_i[T_o]}\; t + \frac{1}{4}\left(\frac{k_p}{\sqrt{k_t}}[LH]\right)^2 k_i t$$





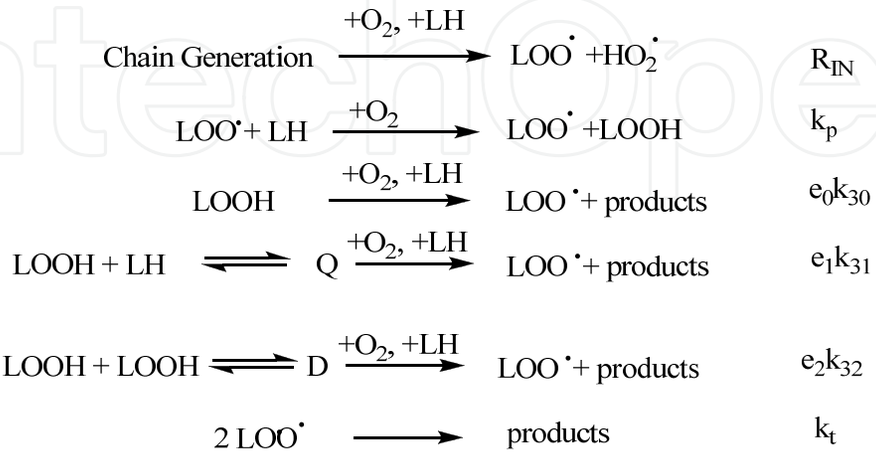


$$[T] = [LOOH] + [Q] + [D] = [LOOH](1 + K_1[LH] + K_2[LOOH])$$

LH: is linoleic acid with its allylic hydrogen
LOO•: peroxide radical
LOOH: lipid hydroperoxides
K_1 and K_2: are the equilibrium constants for complexes Q and D, respectively
[T]: summary concentration of LOOH
k_{30}, k_{31} and k_{32}: are the corresponding rate constants
e_0, e_1, e_2: are the corresponding radicals yield

Scheme 1. Kinetic scheme of lipid hydroperoxide decomposition reactions

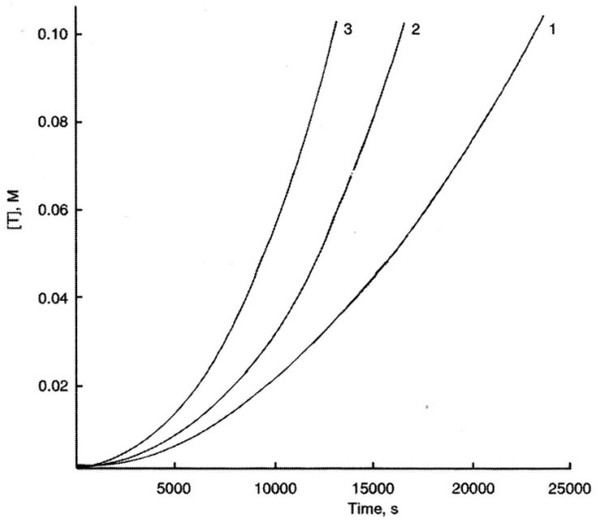
The kinetic scheme 2 is significantly simplified and readily solved assuming a quasi-steady-state for LOO•, rapid achievement of equilibrium and neglected of the loss of Q and LOOH since their decomposition rate constants are low. There are marked: **C** - is the ratio between the equilibrium constants of bi- and pseudo-mono-molecular mechanisms of LOOH decomposition, needed to be marked for the solution of the equation.



R_{IN}: the rate of chain generation, k_p: rate constants of chain propagation, k_t: rate constants of chain

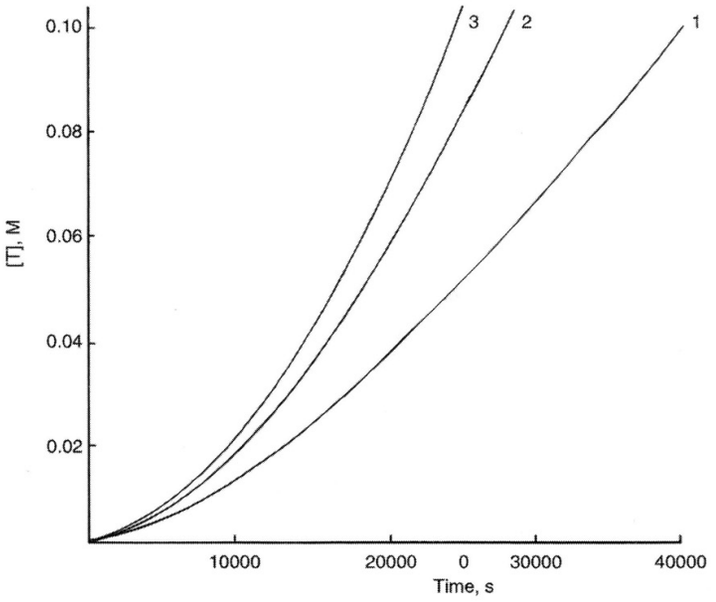
Scheme 2. Kinetic scheme of lipid autoxidation by Kancheva and Belyakov [22]

Figures 2-5 presents kinetics of different mechanisms of lipid hydroperixides decomposition. In Figure 6 it is shown, that k_i doesn't change with growing of MeLi concentration from 0.3 to 1.7 M, when the concentration of MeLi hydroperoxydes is smaller than 5 10^{-3} M. It is established, that MeLi hydroperoxides decay is in agreement with a first order reaction and pseudo-mono-molecular mechanism (a reaction between hydroperoxides and non-oxidized lipid substrate; LOOH + LH).



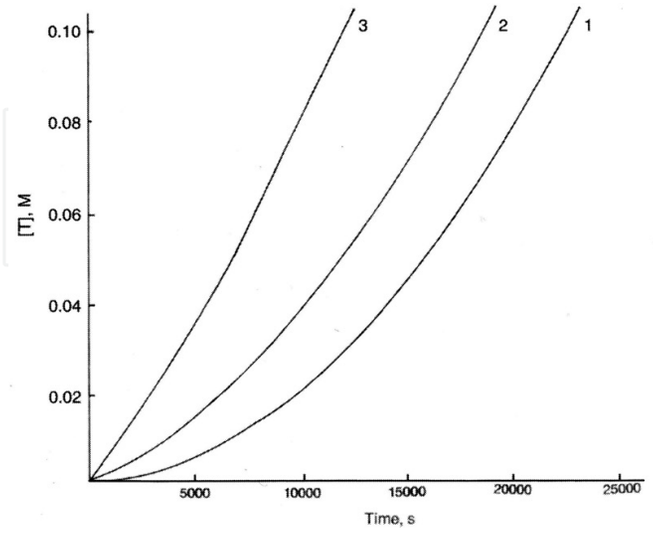
No	W_i, M/s ($\times 10^{-8}$)	W_i^0, M/s ($\times 10^{-11}$)	$(ek_3)_{eff}$ ($\times 10^{-7}$)	c
1	2.0	2.0	2.0	10^{-4}
2	6.3	2.0	4.7	10^{2}
3	8.6	2.2	7.6	10^{3}

Figure 2. Influence of dimer formation equilibrium constant (K_2) on the kinetics of MeLi autoxidation at 60°C, when e_2k_{32} has a great value (e_2k_{32}=2 10^{-6}), [T_0]=10^{-4}M, d=2 10^{-7} and $k_p/(k_t)^{1/2}$ = 6 10^{-2}



No	c	$(ek_3)_{eff}$ ($\times 10^{-7}$)
1	10^3	5.54
2	10^2	1.25
3	20	1.71

Figure 3. Influence of dimer formation equilibrium constant (K_2) on the kinetics of MeLi autoxidation at 60°C, when e_2k_{32} has a small value ($e_2k_{32}= 10^{-10}$), $[T_0]=10^{-4}$M, d=2 10^{-7} and $k_p/(k_t)^{1/2} = 6\ 10^{-2}$(i.e. very small value of e_2)



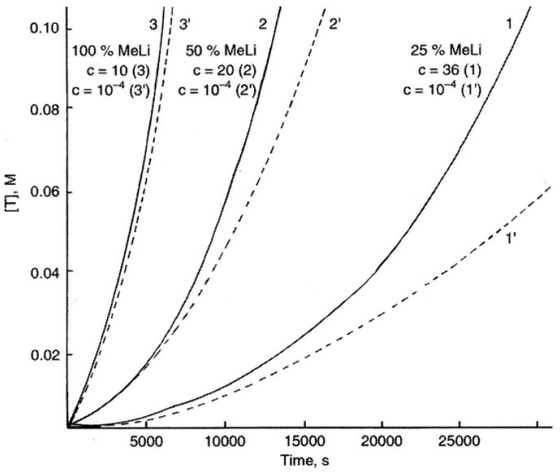
$$\frac{[T]-[T_0]}{t} = A \cdot t + B$$

$$A = \frac{1}{4}\left(\frac{k_p}{\sqrt{k_6}}[LH]\right)^2 \cdot d$$

$$B = \frac{k_2}{\sqrt{k_6}}[LH]\sqrt{W_i^0 + d \cdot [T_0]}$$

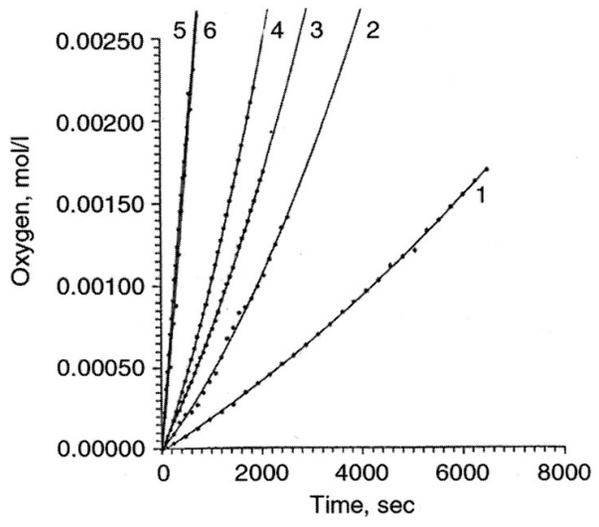
No	W_i^0, M/s	A ($\times 10^{-10}$)	B
1	0	1.8	$2.68\ 10^{-7}$
2	10^{-9}	1.8	$1.92\ 10^{-6}$
3	10^{-8}	1.8	$6.01\ 10^{-6}$

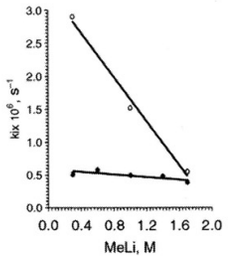
Figure 4. Influence of the substrate (MeLi) diluting with an inert solvent (concentrations of 25, 50 and 100%) at 60°C (e_2k_{32}=2 10^{-6})



No	[LH] MeLi %	c	d $(ek_3)_{eff}$ ($\times 10^{-7}$)	$\frac{k_p}{\sqrt{k_t}}[LH]$	W_i [T] = 0.1 M ($\times 10^{-8}$)
1	25	36	1.3	$4.0\ 10^{-2}$	4.5
1'	25	10^{-4}	3.0	$4.0\ 10^{-2}$	1.3
2	50	20	3.1	$8.0\ 10^{-2}$	4.1
2'	50	10^{-4}	2.0	$8.0\ 10^{-2}$	2.0
3	100	10	3.2	$1.6\ 10^{-1}$	3.8
3'	100	10^{-4}	2.7	$1.6\ 10^{-1}$	2.7

Figure 5. Kinetic curves of inhibited oxidation and autoxidation of MeLi at 60°C, when there is no dimerization of lipid hydroperoxides (K_2=0)





$$LOOH + LH \overset{K_1}{\rightleftharpoons} Q \qquad LOOH + ROH \overset{K_S}{\rightleftharpoons} S$$

No	[MeLi] M	[1 – OD] M
1	0.3	0
2	0.3	0.1
3	1.0	0
4	1.0	0.1
5	1.7	0
6	1.7	0.1

Figure 6. Effect of ROH (0.1M, 1-Octadecanol, 1-OD) on the kinetics of hydroperoxide accumulation of MeLi at 60°C, at different MeLi concentrations (0.3, 1.0 and 1.7M) and dependence of the effective initiation rate constants k_i on [LH] in the absence (1) and in presence of 0.1M 1-Octadecanol (2).

It is shown, that in presence of a lipid hydroxyl compound k_i^{ROH} is strongly growing with the decrease of MeLi (LH) concentration (Figure 6). This is explained with the competition of reactions (LOOH + LH and LOOH + ROH). Some different mechanisms, which are possible for reaction between LOOH and ROH, were discussed (Scheme 3).

$$LOOH + ROH \underset{}{\overset{K_S}{\rightleftharpoons}} S$$

$$[S] = K_s[LOOH][ROH]$$

$$S \xrightarrow{+O_2} LOO^{\cdot} + \text{products} \qquad e_s k_s$$

$$[T]=[LOOH]+[Q]+[S] =[LOOH](1+K_1[[LH]+K_s[ROH])$$

$$[LOOH] = [T]/(1+K_1[LH] +K_s[ROH])$$

$$[Q] = K_1[LH]\,[T]/(1+K_1[LH]+K_s[ROH]$$

$$[S] =K_s[ROH]\,[T]\,/(1+K_1[LH]+K_s[ROH])$$

Ks: is the equilibrium constant for complex S, initial rate of decomposition of complex S ($e_s k_s$)
Lipid hydroxy compounds (LOH) from the oxidized lipid substrate (LH) is formed during the whole oxidation process:
LOH and H_2O with the rate $e_0\, k_{30}$ [LOOH] from the hydroperoxides of substrate LH
LOH and H_2O with the rate $e_1\, k_{31}$ [Q] from the Q|
LOH and H_2O with the rate $e_3\, k_{33}$ [S] from the S

Scheme 3. Kinetic scheme of lipid hydroperoxides (LOOH) decay in presence of ROH

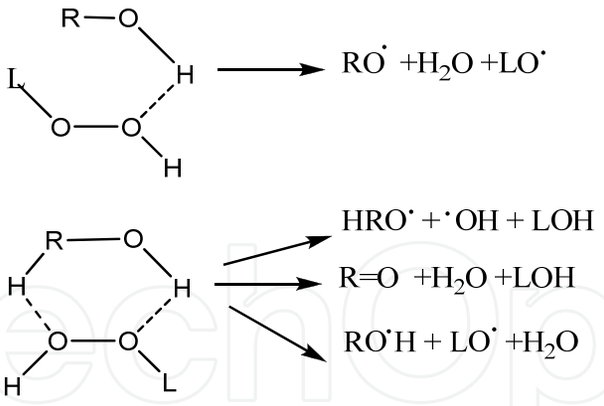
$$A = \frac{1}{4}\left(\frac{k_p}{\sqrt{k_t}}[LH]\right)^2 k_i$$

$$k_i = \frac{4A}{(\frac{k_p}{\sqrt{k_t}}[LH])^2}$$

$$B = \frac{k_p}{\sqrt{k_t}}\sqrt{k_i[T_o]}$$

$$[T_o] = (\frac{B}{\frac{k_p}{\sqrt{k_t}}[LH]\sqrt{k_i}})^2$$

There are presented some different mechanisms of the interaction between lipid hydroperoxides (LOOH) and hydroxy compounds (ROH):



Scheme 4. Additional lipid hydroperoxides decomposition in presence of an antioxidant (AH)

$$LOOH + AH \underset{}{\overset{K_P}{\rightleftharpoons}} P \xrightarrow[LH,\ O_2]{k_{iP}} LO_2^{\cdot}$$

$$K_P = \frac{[P]}{[LOOH][AH]}$$

K_P: the equilibrium constant for complex P,
k_{iP}-initiation rate constant of P decomposition
Total hydroperoxides concentration [T] in presence of ROH and AH
[T] = [LOOH]+[Q]+[S]+[P]= [LOOH](1+K1[LH]+KS[ROH]+KP[AH])

Scheme 5. Equilibrium constant of complex formation between an antioxidant (AH) and lipid hydroperoxides (ROH)

$$AH + ROH \underset{}{\overset{K_0}{\rightleftharpoons}} \left[AH\ldots O \begin{matrix} H \\ \diagdown \\ \diagup \\ R \end{matrix} \right]$$

$$K_0 = \frac{[AH]_0 - [AH]}{[AH][ROH]}$$

$$[AH] = \frac{[AH]_0}{1 + K_0[ROH]}$$

Scheme 6.

It has been proven [23,24] that fatty alcohols with different chain length, mono- and diacylglycerols increase the rate of LOOH decomposition into free radicals and thus accelerated lipid oxidation in absence of an antioxidant. In presence of phenolic antioxidants ROH make complexes basing on H bond formation and thus decrease the antioxidant efficiency of them [25,26]. DL-alpha –tocopherol and butylated hydroxyl toluene demonstrate the best antioxidant efficiency in presence of ROH [26]. Taking into account that ROH are formed during the proceeding, transportation and storage of lipids and lipid containing products as a result of hydrolysis, it is of importance to know how to improve their oxidative stability.

4. Antioxidants – Inhibitors of lipid oxidation

The introduction of antioxidants in the affected body normalizes not only the peroxide oxidation, but also the lipid content. Antioxidants used in oncology are effective in the first stages as mono-therapy with antioxidants at high concentrations and at the last stages mainly as additives in the complex tumor therapy - the antioxidant is in low concentrations. In this respect the medical treatment of most of diseases includes formulations based on a combination of traditional drugs with targeted functionality and different antioxidants [3,27].

The activity of antioxidants depends on complex factors including the nature of the antioxidants, the condition of oxidation, the properties of substrate, being oxidized and the stage of oxidation [2,3,27-33].

Capacity of antioxidants has at least two sides: the antioxidant potential, determined by its composition and properties of constituents and is the subject of food chemistry, and the biological effects, depending, among other things, on bioavailability of antioxidants, and is a medico-biological problem.

4.1. Classification of antioxidants [2,16,30-36]

Depending on their mechanism of action:

Antioxidants, inhibiting lipid oxidation by trapping lipid peroxide radicals– they are aromatic compounds with a weak O-H, N-H bonds (phenols, amines, aminophenols, diamines etc.

Antioxidants, inhibiting the oxidation process by trapping alkyl radicals – they are quinones, methylene quinones, which are effective in low oxygen concentration.

Hydroperoxide decomposers – these compounds react with hydroperoxides without formation of free radicals.

Metal chelators – oxidation process can be inhibited by addition of compounds, forming complexes with metal ions and thus made them inactive towards hydroperoxides. In this groups are hydroxyl acids, flavonoids etc.

Antioxidants with multistage action – systems containing such kind of compounds (alcohols and amines) inhibitors can be regenerated during the oxidation process.

Inhibitors with combined action – inhibitor molecule has two or more functional groups, each of them react in different reactions.

Depending on their nature:

Natural antioxidants – usually with low toxicity (with some exception), wide spectrum of biological and antioxidant activities.

Synthetic - they are with a high antioxidant activity. However, antioxidants for application in foods and additives or supplements they must pass additional criteria (no toxicity, safety, healthy, low cost, etc.)

Depending of their biological activity:

Bio-antioxidants – compounds with both biological and antioxidant activities. Last decades there is a growing interest to the nature-like bio-antioxidants. The most important known bio-antioxidants are flavonoids and phenolic acids.

Antioxidants without a biological activity - some even natural antioxidants can show a toxic activity and for that reason they must be tested.

Depending on the number of phenolic groups:

Monophenols – the known compounds are butylated hydroxyl toluene (BHT), Tocopherol (TOH), p-coumaric acid (p-CumA), ferulic acid (FA), sinapic acid (SA) etc.

Biphenols – the known compounds are caffeic acid (CA), hydroquinone (HQ), tertbutylated hydroquinone (TBHQ) etc.

Polyphenols –flavonoids: quercetin (Qu), rutin (Ru), luteolin (Lu), kampferol (Kf), isorhamnetin (Isorh) etc.

The inherent compositional and structural complexity of real foods and in vivo studied means that systematic studies of lipid oxidation must first be carried out in model systems. The following models were applied to explain the structure-activity relationship of different phenolic antioxidants: model 1, a DPPH assay used for the determination of the radical scavenging capacity (AH+DPPH• ⟶ A·+DPPH-H); model 2, chemiluminescence (CL) of a model substrate RH (cumene and diphenylmethane) used for determination of the rate constant of a reaction with model peroxyl radicals (AH+RO_2·⟶ A·+ROOH); model 3, lipid autoxidation (LAO) used for the determination of the chain-breaking antioxidant efficiency and reactivity (AH+LOO·⟶ A·+LOOH; A·+LH(+O_2)⟶ AH+LOO·); and model 4, theoretical methods used for predicting the activity (predictable activity by statistical and/or quantum-chemical calculations).

4.2. Structure of the antioxidants

By combination of different experimental methods: DPPH test, lipid autoxidation kinetics, chemiluminescence kinetics and quantum chemical calculations it has been proven that the prooxidant activity of chalcones is due to the possible reaction of phenoxyl radicals formed with oxygen and formation of dioxiethanes, [37]Vasil'ev *et al.*, 2009:

New bis-coumarins are found to have anti-HIV activity [38]. Together with their antioxidant capacity (Fig. 8C) they are one of the most important bio-antioxidants nowadays.

The studied simple dihydroxy-coumarins are natural (Cum0) and nature-like synthetic compounds with a wide range of biological activities against cancer, inflammatory, cardio-vascular diseases, diabetes etc. Together with the strong antioxidant activity and synergistic effect with Tocopherol, they are very important for the practical application, [40]Kancheva *et al.*, 2010a.

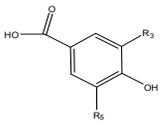
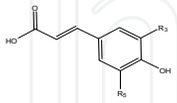
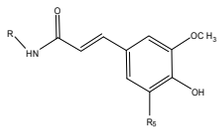
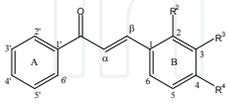
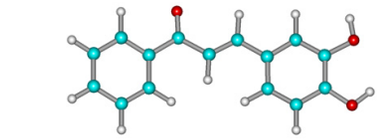
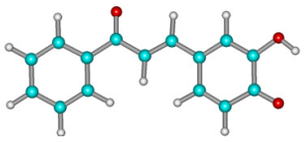
A. Benzoic acids	Abbr.	R3	R5	Activity	Methods
	HBA	H	H	Weak	LAO,CL,Theor
	VanA	H	OCH_3	Weak	LAO,CL,Theor
	SyrA	OCH_3	OCH_3	Moderate	LAO,CL,Theor
	DHBA	OH	H	Strong	LAO,CL,Theor
	GA	OH	OH	Strong	LAO, DPPH,Theor
B.Cinnamic acids	p-CA	H	H	Weak	LAO, CL,DPPH, Theor
	FA	OCH_3	H	Moderate	LAO, CL,DPPH, Theor
	SA	OCH_3	OCH3	Moderate	LAO, CL,DPPH, Theor
	CA	OH	OH	Strong	LAO, CL, DPPH, Theor.
	PHC	Prenyl	H	Strong	LAO
	DPHC	Prenyl	Prenyl	Moderate	LAO
C. N-cinnamic acids amides	Abbr.	R	R5	Activity	Method
	N1	$CH(CH_2C_6H_5)$-$COOC(CH_3)_3$	H	Moderate	LAO
	N2	$CH(CH_2C_6H_4$-F-m)-$COOCH_3$	H	Moderate	LAO
	N3	$CH(CH_3)$-$COOC(CH_3)_3$	H	Moderate	LAO
	N4	$CH(CH_2C_6H_4$-OH-p)-$COOCH_3$	H	Moderate	LAO
	N5	$CH(CH_2C_6H_4$-OH-p)-$COOCH_3$	OCH_3	Strong	LAO
	N6	$CH(CH_2C_6H_4$-F-m)-$COOCH_3$	OCH_3	Strong	LAO
	N7	$CH(CH_2C_6H_5)$-$COOC(CH_3)_3$	OCH_3	Strong	LAO
D.Hydroxy-chalcones	Abbr.	R2	R3 R4	Activity	Methods
	Ch1	H	OH H	Weak	LAO, CL, DPPH, Theor
	Ch2	H	H OH	Moderate	LAO, CL, DPPH,Theor
	Ch3	H	OH OH	Strong	LAO, CL, DPPH Theor
	Ch4	H	OH OCH_3	Weak	LAO, CL, DPPH,Theor
	Ch5	OH	H H	Weak	LAO, CL, DPPH,Theor
	Ch6	OH	OCH_3 H	Weak	LAO, CL, DPPH,Theor

Table 3. The main structures and activities with methods for benzoic acids, cinnamic acids, N-cinnamic acids amides and chalcones

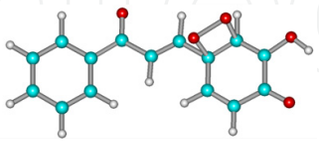


Chalcone Ch3(ΔH_f^0 = -58.3 kcal/mol)

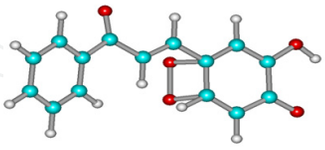


Radical formed from Ch 3 (ΔH_f^0 = -29.0 kcal/mol)

(a)



D1



D2

Dioxiethanes D1 and D2
$\Delta H_f^0(D_1^{\bullet})$ = -19. 9 kcal/mol, $\Delta H_f^0(D_2^{\bullet})$ = -27.1 kcal/mol

(b)

Figure 7. a) Optimized structures of Chalcone Ch3 (7a) and its aryl radical (7b); b) Optimized structures of Dioxiethanes D1 and D2 formed from Ch3 radical and oxygen.

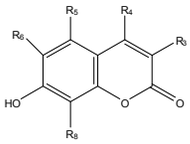
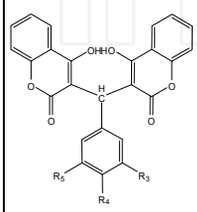
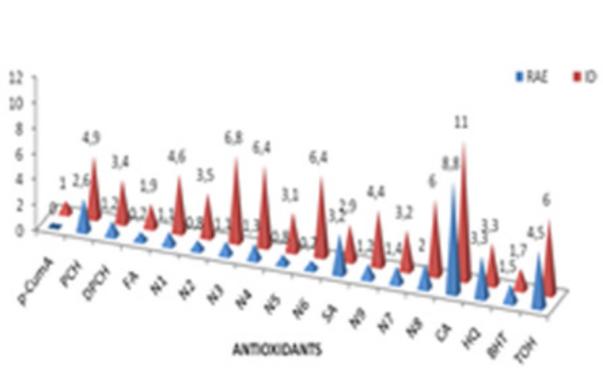
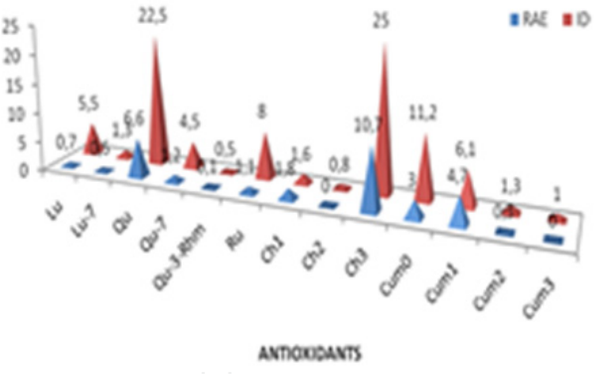
E.Simple Coumarins	**Abbr.**	**R3**	**R4**	**R5**	**R6**	**R8**	**Activity**	**Method**
	Cum0	H	H	H	OH	H	Strong	LAO
	Cum1	H	CH_3	H	OH	H	Strong	LAO
	Cum2	H	CH_3	H	H	OH	Strong	LAO
	Cum3	EtCOOMe	CH_3	H	H	OH	Strong	LAO
	Cum4	MeCOOEt	CH_3	H	H	OH	Strong	LAO
	Cum5	H	CH_3	OH	H	H	Weak	LAO
	Cum6	H	CH_3	H	H	H	Weak	LAO
	Cum7	H	OH	H	H	H	Weak	LAO
F.Bis-Coumarins	**Abbr.**	**R3**	**R4**	**R5**	**Activity**	**Method**	**Abbr.**	**R3**
	Bis-Cum1	OH	OH	H	Strong	LAO	Bis-Cum1	OH
	Bis-Cum2	OCH_3	OH	OCH_3	Moderate	LAO	Bis-Cum2	OCH_3
	Bis-Cum3	OCH_3	OH	NO_2	Weak	LAO	Bis-Cum3	OCH_3
	Bis-Cum4	OCH_3	OCH_3	H	Weak	LAO	Bis-Cum4	OCH_3
	Bis-Cum5	OCH_3	OCH_3	OCH_3	Weak	LAO	Bis-Cum5	OCH_3

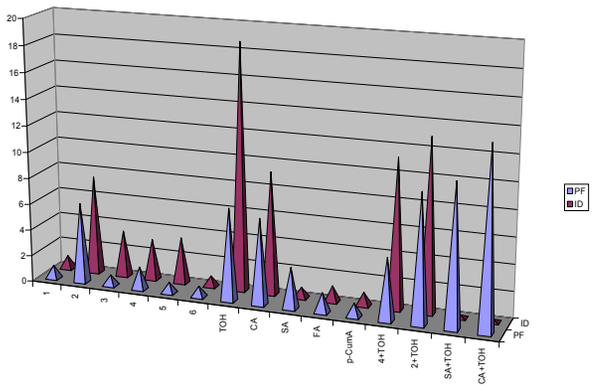
Table 4. Simple and Bis-Coumarins



(a) [30]Kancheva, 2009

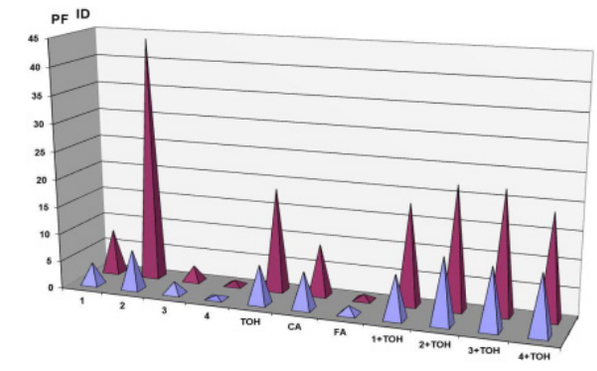


(b) [30]Kancheva, 2009



1=Cum7; 2-6 corresponding bis-coumarins

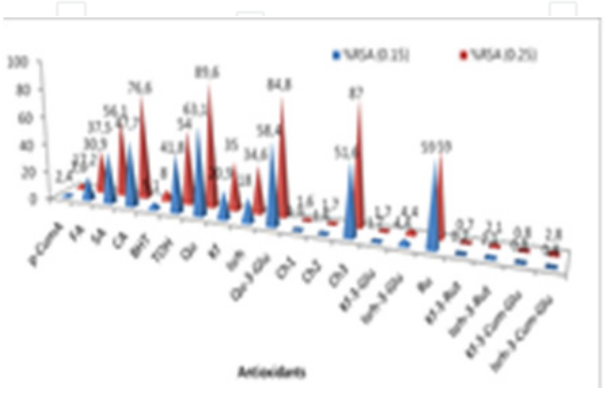
(c) [38]Kancheva et al, 2010b



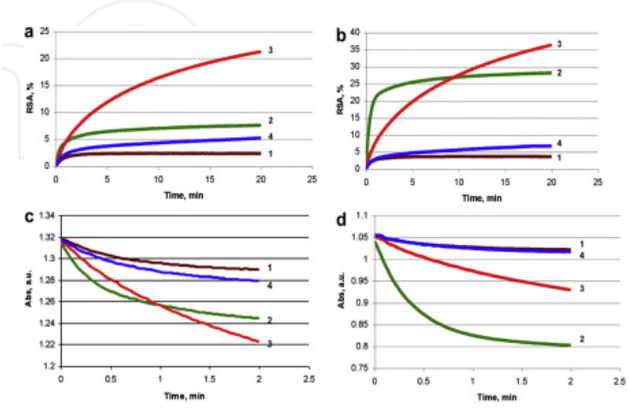
Xanthene(1,2) and neo-lignans (3,4)

(d) [39]Kancheva et al, 2012

Figure 8. The main kinetic parameters PF, RAE (antioxidant efficiency) and ID (inhibition degree) of lipid autoxidation in presence of different antioxidants (for abbreviation see corresponding tables)



(a) [30] Kancheva, 2009
[DPPH]/[AH]=0.15 and 0.25,



(b) [39]Kancheva et al, 2012
[DPPH]/[AH]=0.25 (a,c) and 0.40 (b,d)

Figure 9. Radical scavenging activity (%) of studied compounds at different [DPPH]/[AH] ratio

Nature-like neo- and xhanthene-lignans recently synthesized showed activity agains cardio-vascular, inflammatory and cancer diseases. Together with their excellent capacity to scavenge free radicals and to inhibit lipid autoxidation these bio-antioxidants are of great importance for the practie, as individuals and in binary mixtures with TOH [39].

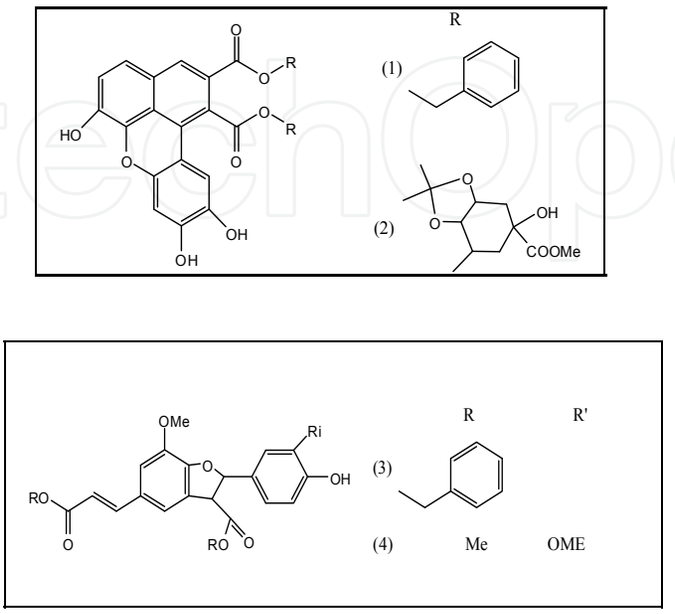


Figure 10. Structures of Xanthene (MF1, MF2) and neo-lignans (MF3, MF4)

The highest values of radical scavenging activity (%RSA$_{max}$) and largest rate constants for reaction with DPPH radical were obtained for xanthenes and neo-lignans (compounds 2 and 3, Fig. 7B). Comparison of %RSA$_{max}$ with that of standard antioxidants DL-a-tocopherol (TOH), caffeic acid (CA) and butylated hydroxyl toluene (BHT) give the following new order of %RSA max: TOH(61.1%) > CA(58.6%) > 3(36.3%) > 2(28.1%) > 4(6.7%) > 1(3.6%) = BHT(3.6%). On the basis of a comparable kinetic analysis with standard antioxidants a new order of the antioxidant efficiency were obtained: **PF:** 2(7.2) ≥ TOH(7.0) > CA(6.7) > 1(3.1) > 3(2.2) > FA(1.5) > 4(0.6); and of the antioxidant reactivity: **ID:** 2(44.0) >> TOH(18.7) >> CA(9.3) >> 1(8.4) > 3(2.8) >FA(1.0) > 4(0.9) [36].

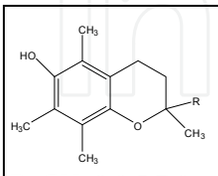
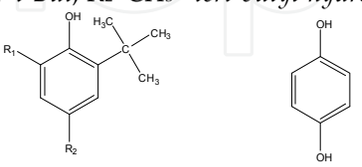
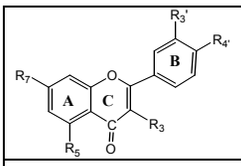
 *R=CH$_3$ 2,2,5,7,8-pentamethyl-chroman-3-ol (Chroman C1)* *R=Phytyl; alpha-tocoperol (TOH)*	*R$_1$=H; R$_2$=OH –tert-butylated-hydroquinone (TBHQ)* *R$_1$=t-But; R$_2$=CH$_3$ –tert-butyl-hydroxytoluene (BHT)*  *Hydroquinone (HQ)*
ChrC1 and TOH -Strong activity, LAO, CL, DPPH,Theor	*TBHQ, HQ -strong activity, BHT –weak/moderate activity; LAO, CL, DPPH,Theor*

Table 5. Standard Antioxidants

	**R3′**	**R4′**	**R3**	**R5**	**R7**	**%RSA exper**	**%QSAR theor**	**LAO Activity**
Quercetin (Qu)	OH	OH	OH	OH	OH	62.2	88.40	Strong
Qu-3-O-Glu	OH	OH	O-Glu	OH	OH	63.9	88.40	Strong
Qu-3-O-Rhm	OH	OH	O-Rhm	OH	OH	59.0	88.40	Strong
Qu-3-O-Rut	OH	OH	O-Rut	OH	OH	62.2	88.40	Strong
Qu-7-O-Glu	OH	OH	OH	OH	O-Glu	nd	nd	Strong
Luteolin (Lu)	OH	OH	H	OH	OH	nd	nd	Strong
Lu-7-O-Glu	OH	OH	H	OH	O-Glu	nd	nd	Strong
Kampferol (Kf)	H	OH	OH	OH	OH	54.4	88.40	Strong
Kf-3-O-Glu	H	OH	O-Glu	OH	OH	1.7	12.75	Weak
Kf-3-O-Rut	H	OH	O-Rut	OH	OH	0.7	12.75	Weak
Kf-3-O-Cum-Glu	H	OH	O-Cum-Glu	OH	OH	0.8	12.75	Weak
Isorhamnetin (Isorh)	OCH3	OH	OH	OH	OH	19.2	88.40	Strong
Isorh-3-O-Glu	OCH3	OH	O-Glu	OH	OH	4.4	12.75	Weak
Isorh-3-O-Rut	OCH3	OH	O-Rut	OH	OH	2.1	12.75	Weak
Isorh-3-O-Cum-Glu	OCH3	OH	O-Cum-Glu	OH	OH	2.8	12.75	Weak

Glu: D-glucoside; Rut: rutinoside; Glu-Com: p-coumaroyl-glucosides;
%QSARtheor=3.954+75.950.$I_{3',4'\text{-di-OH or 3-OH}}$ + 8.499.$I_{5\text{-OH}}$ – by statistical analysis (QSAR) of Amic *et al* [43]
(I=1 for 3′,4′-di-OH and/or3-OH) and I=1 for 5-OH); %QSAR=3.95+8.5+75.95 (for Qu all derivatives, Kf, Isrh) – 88.40;
%QSAR=3.95+8.5 (for Kf 3Oderivatives and Isrh 3Oderivatives) – 12.75

Table 6. Substitution pattern of the series of flavonoids examined for their radical scavenging activity [41-43]

4.3. Synergism, additivism and/or antagonism of binary mixtures of phenolic antioxidants [30,31,35-37,41-45]

It is known that in the literature usually are published data about mixtures without or with synergism between the components. Separation of different effects of binary mixtures (synergism, additivism and/or antagonism) of different antioxidants was made for the first time by Denisov [32]. The latest gives possibility to make differences about different effects of binary mixtures, not only to be separated as mixtures without or with a synergism.

Synergism – is observed when the inhibiting effect of the binary mixtures (IP_{1+2}) is higher than the sum of the induction periods of the individual phenolic antioxidants ($IP_1 + IP_2$) i.e. $IP_{1+2} > IP_1 + IP_2$. The percent of the synergism is presented by the following formulae % *Synergism* = $100[IP_{1+2} – (IP_1 + IP_2)]/ (IP_1 + IP_2)$.

Additivism - is observed when the inhibiting effect of the binary mixtures (IP_{1+2}) is equal to the sum of the induction periods of the phenolic antioxidants alone ($IP_1 + IP_2$) i.e. $IP_{1+2} = IP_1 + IP_2$.

Antagonism - is observed when the inhibiting effect of the binary mixtures (IP_{1+2}) is lower than the sum of the induction periods of the individual phenolic antioxidants ($IP_1 + IP_2$) i.e. $IP_{1+2} < IP_1 + IP_2$.

Binary mixtures (1:1)	***[AH] mM***	***IP_{1+2} h***	***IP_1 h***	***IP_2 h***	***Effects, %***	***Ref***
Qu (1) + Lu(2)	0.1	7.5±0.8	9.9±0.9	2.2±0.2	Antagonism	41
	0.5	12.3±0.9	24.5±0.6	6.3±0.4	Antagonism,	41
Qu (1) + Ru(2)	0.1	8.3±0.8	9.9±0.9	2.7±0.2	Antagonism	41
	0.5	21.5±0.6	24.5±0.6	2.8±0.2	Antagonism	41
Qu-7(1) + Lu-7(2)	0.5	2.0±0.2	1.5±0.2	1.8±0.2	Antagonism	41
	1.0	2.1±0.2	1.0±0.2	1.4±0.2	Additivism	41
Qu (1)+ α-TOH(2)	0.1	29.7±1.5	9.9±0.9	10.5±0.9	Synergism,46%	41
Ru (1) + α-TOH(2)	0.1	24.9±1.5	2.7±0.2	10.5±0.9	Synergism,87%	41
#Myr (1) +α-TOH(2)	0.1	10.5±0.9	4.7±0.3	3.2±0.2	Synergism,33%	3
	0.3	20.5±1.5	8.9±0.9	5.5±0.5	Synergism,42%	3
	0.6	31.1±1.5	16.3±0.9	7.4±0.5	Synergism,14%	3
CA (1) + α-TOH(2)	0.1	20.4±1.5	9.8±0.9	10.5±0.9	Additivism	3
SA(1) +α- TOH(2)	0.1	16.1±0.9	5.3±0.5	10.5±0.9	Additivism	3
BHT(1) +α- TOH(2)	0.1	21.5±1.5	7.5±0.5	10.5±0.9	Synergism,19%	3
TBHQ(1) + αTOH(2)	0.1	26.1±1.5	7.9±0.5	10.5±0.9	Synergism,42%	3
Cum_1(1) + αTOH(2)	0.1	11.8±0.9	1.5±0.2	10.5±0.9	Additivism	40
Cum_6 (1) + αTOH(2)	0.1	14.2±0.9	2.0±0.2	10.5±0.9	Synergism,14%	40
Cum_4 (1) + αTOH(2)	0.1	12.7±0.9	7.1±0.5	10.5±0.9	Antagonism	40
BisCum1(1) + αTOH(2)	0.1	12.6±0.9	7.9±0.5	10.5±0.9	Antagonism	38
BisCum3(1) + αTOH(2)	0.1	6.1±0.5	2.2±0.2	10.5±0.9	Antagonism	38
MF1(1)+α-TOH(2)	0.1	10.0±0.9	4.0±0.3	10.5±0.9	Antagonism	39
MF2(1)+α-TOH(2)	0.1	15.0±0.9	9.2±0.9	10.5±0.9	Antagonism	39
MF3(1)+α-TOH(2)	0.1	14.0±0.9	2.8±0.2	10.5±0.9	Synergism,5.3%	39
MF4(1)+α-TOH(2)	0.1	13.8±0.9	0.75±0.05	10.5±0.9	Synergism,22%	39
#SA(1) + α-TOH(2)	0.1	45.0±1.0	8.5±0.5	21.0±1.5	Synergism,52%	44
Lipid substrate oxidized TGSO, 80°C , only # TGL,100°C						

Table 7. Effects of equimolar (1:1) binary mixtures of studied antioxidants without and with alpha-tocopherol (α-TOH)

Syn*er*gism obtained for different binary mixtures are explained taking into account that the during the oxidation process the antioxidant molecules of both strong antioxidants may be regenerated, which leads to higher antioxidant efficiency of the mixture, than of the individual compounds. The regeneration of both antioxidant molecules of compounds with catecholic moiety, QH_2 (4-hydroxy-bis-coumarin, caffeic acid (CA) and MF1-MF3) and of tocopherol (TOH) is possible as a result of the following possible reactions:

a. reaction of an antioxidant radical ($QH^{\bullet}$ or $TO^{\bullet}$) with other antioxidant molecule:
- semiquinone radical ($QH^{\bullet}$) and tocopherol (TOH)

$QH^{\bullet} + TOH \rightarrow QH_2 + TO^{\bullet}$ (regeneration of QH_2 by H transfer)

- between tocopheryl radical ($TO^{\bullet}$) and QH_2

$TO^{\bullet} + QH_2 \rightarrow TOH + QH^{\bullet}$ (regeneration of TOH by H transfer)

b. homo-disproportionation reaction of two equal radicals:

$2QH^{\bullet} \rightarrow QH_2 + Q$ regeneration of QH_2 (Q is quinone)

$2TO^{\bullet} \rightarrow TOH + T{=}O$ regeneration of TOH (T=O is tocopheryl quinone)

c. cross-disproportionation reaction of different radicals:

$QH^{\bullet} + TO^{\bullet} \rightarrow QH_2 + T{=}O$ regeneration of QH_2

$TO^{\bullet} + QH^{\bullet} \rightarrow TOH + Q$ regeneration of TOH.

As a result the oxidation stability of lipid sample increases, because the both antioxidants with strong efficiency are regenerated during the oxidation process.

In case of binary mixture of TOH with monophenolic antioxidants (AH), predominantly TOH molecule will be regenerated during the following reactions:

- tocopheryl radical ($TO^{\bullet}$) with monophenolic antioxidant (AH)

$TO^{\bullet} + AH \rightarrow TOH + A^{\bullet}$

- homo-disproportionation of tocopheryl radicals ($TO^{\bullet}$)

$2TO^{\bullet} \rightarrow TOH + T{=}O$

- cross-disproportionation of phenoxyl radical ($A^{\bullet}$) and tocopheryl radical ($TO^{\bullet}$)

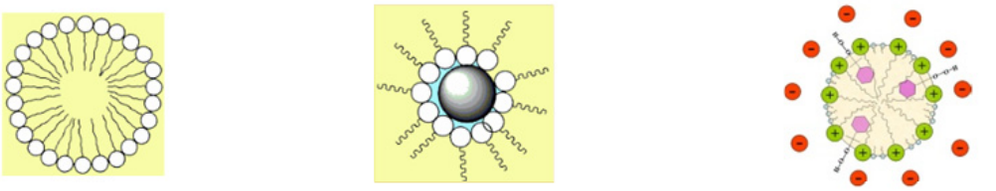
$A^{\bullet} + TO^{\bullet} \rightarrow TOH + A\text{-}H$ or $A^{\bullet} + TO^{\bullet} \rightarrow T{=}O + AH$ (depending on AH structure)

These reactions demonstrate that during oxidation process initial molecules of individual antioxidants are regenerated by different mechanisms in the binary mixtures. Nevertheless both binary mixtures may be used as effective antioxidant compositions. It is proven that the positions of phenolic hydroxyl groups in 4-hydroxy-bis-coumarins are of significance for their antioxidant activity and mechanism of action. Comparable kinetic analysis showed that the antioxidant efficiency (PF) and reactivity (ID) depend significantly from the substitution of the phenolic ring.

5. Surface-active compounds – surfactants (S)

Surfactants (S) are amphiphilic substances which adsorb at interface and decrease an excess of free energy (surface tension, γ) of interface. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. A surfactant molecule contains both a water insoluble hydrophobic component and a water soluble hydrophilic component (polar head).

Surfactant solutions are one of the simplest examples of self-assembling soft nano-systems, whose micro-aggregates (micelles) are of 1–500 nm in size [10,11]. Micelles are prevalent in naturally occurring and biological catalytic reactions Micelles are formed by those surfactants which possess rather long bulky hydrophobic part along with strong hydrophilic head. Such surfactants form direct micelles in water and other polar solvent, and reverse micelles in organic solution.



(a) Direct micelle in water (b) Reverse micelle in oil (c) Mixed micelle {nROOH…mS}

Figure 11. Micelles formed by surfactants in polar and nonpolar media

The phenomenon of micellar catalysis has been known for a rather long time and applied in many processes, but the significant influence of surfactants on the lipid and hydrocarbon oxidation has been found and studied only in recent decades [6-8, 46-48]. Specific features of micellar catalysis for oxidation processes have two causes: (1) Hydroperoxides (LOOH), which are formed as the primary oxidation products, are amphiphilic and surface active, in contrast to initial oils; (2) There is spontaneous allocation of amphiphilic compounds in every heterogeneous and colloid system resulting in the reduction in the total free energy of a system, including the interface boundaries (the rule of polarity equalization). In the presence of surfactants in oxidized oil hydroperoxides and surfactants form mixed micelles {nLOOH...mS}(Fig. 11c). Using the measurement of the interphase tension [49], nuclear magnetic resonance (NMR), and dynamic light scattering [50], it was shown the association of LOOH and a surfactant in combined micelles in which LOOH plays the role of a co-surfactant. The average self-diffusion coefficient for hydroperoxide decreases with growth of the surfactant (CTAC) concentration up to the equalization with the surfactant diffusion coefficient, when all LOOH is bound in mixed micelles {nLOOH···mS}. The mixed micelle effective size calculated by the Stocks–Einstein equation was ~2 nm [50]. The size determined by DLS for mixture cumene hydroperoxide and CTAB are about 20 nm. Hydroperoxide facilitates the colloid dilution of CTAB in organic medium.

5.1. Effect of surfactants on lipid oxidation.

The comparison of the effects of different surfactants on lipid and hydrocarbon oxidation reveals that cationic surfactants (CS) promote hydroperoxide destruction resulting in the formation of free radicals [46,47] and the oxidation as a whole (Fig.12a,b).

By means of NMR and GC–MS, it is shown that in the presence of CS (CTAC and CTAB)) cumene hydroperoxide decomposes into dimethyl phenylcarbinol, acetophenon, and dicumylperoxide which are known as resulting from the radical decomposition of hydroperoxide. In the presence of anionic SDS, cumene hydroperoxide decomposes without radical formation into phenol and acetone [48].

The kinetics of oxidation of sunflower (TGSO) and olive (TGOO) oil triacylglycerols (Fig.12a) and natural olefin (limonene) (Fig.12b) in the presence of surfactants show that cationic surfactants (CS) promote the oxidation, whereas the anionic sodium dodecylsulfate (SDS) has no influence in the case

of limonene and SDS demonstrates a weak retardation in TGOO oxidation (Fig.12a.) The chain breaking inhibitor α-tocopherol completely suppress the limonene oxidation accelerated by CTAC and CTAB (Fig.12b). Before and after the induction periods, the oxidation rate is described by the well known equation for the liquid-phase chain oxidation of hydrocarbons and lipids (see above):

$$R_{02} = a\left[LH\right]\cdot R_{IN}^{0.5} \quad (1)$$

where R_{IN} is the radical initiation rate. R_i can be calculated from the duration of the induction period (τ), caused with α-tocopherol (Fig.12) as follows: $R_{IN} = 2[InH]/\tau$.

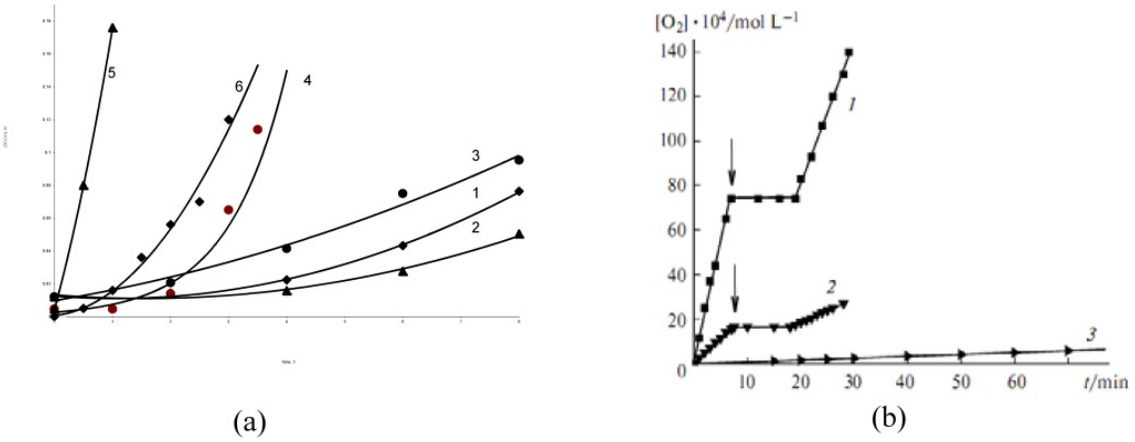


Figure 12. (a). Effect of surfactant on the LOOH formation during autoxidation of TGOO at 100oC and TGSO at 80oC: 1, 4- without additives; 3, 5 - 0.1M CTAB; 2,4- 0.1 M SDS; 6 - 0.04 M 1-OD [48]; (b) Effect of surfactant on the oxygen absorption during 1M limonene oxidation at 60oC; 1 – 1mM CTAC; 2 – 0,8 mM CTAB; 3 – 1mM SDS; 3- without additives; [LOOH]=22mM; Arrows show the moments of introducing α-tocopherol: 1-0,8 mM; 2 – 0,2 mM [51].

The rate constants for the propagation (k_P) and termination (k_t) of the oxidation chain for limonene and its oxidizability parameter $a = k_P/(2k_t)^{0.5}$ are known at the temperatures 30-80°C [52]. So, the radical initiation rate can be calculated on the base of the measured value of oxygen uptake rate: $R_i = (R_{02}/a[LH])^2$. In the case of limonene both formulas give very close R_i values. These data show that the mechanism of the catalytic action of CS on the oxidation processes consists in the increase of the chain initiation rate, caused by acceleration of hydroperoxides decomposition into free radicals.

To estimate the mixed micelles {nLOOH… mS} as a free radical initiator quantitatively, cumene hydroperoxide and hydrogen peroxide, which are produced in industrial scale, and limonene hydroperoxide were taken, and natural polyphenol quercetin was used as a radical acceptor. The interaction of LOOH with quercetin (Qu) in the presence of surfactants (S) is described by reactions:

$nLOOH + mS \circledcirc \{nLOOH \ldots mS\} \rightarrow LO_2^{\cdot}$
$Qu + LO_2^{\cdot} \rightarrow$ products,

The initiation rate is equal to: $R_i = -2d[Qu]/dt$. Quercetin is characterized by an intense absorption band in a visible region of the electronic spectrum, it is soluble in water and organic solvents and readily reacts with free radicals; i.e. it is a convenient kinetic probe to study the processes of radical generation in various media. The generated peroxyl radicals come into the volume and can initiate chain oxidation, polymerization, or other radical processes. In the presence of oxygen the concentrations of hydroperoxide and other polar products increase during the accelerated oxidation, and this, in its turn, influences the structure and properties of micelles. The instability of micelles and also the varied composition of their polar cores do not allow applying the known and frequently used pseudophase approach to the analysis of the lipid oxidation kinetics in the presence of surfactants. The comparison of the activities of LOOH and cationic surfactants in the generation of radicals can be conducted on the basis of the specific rates of radical initiation $\varpi_i = R_i / ([LOOH]\cdot[S])$. Data in the Table 6 show that cationic surfactants catalyze free radical initiation from hydroperoxide both in water and in organic solutions that is both in direct and reverse mixed micelles.

It means that the CS–hydroperoxide system can be used both as a lipophilic and a hydrophilic initiator. Small amounts of LOOH and CS provide significant radical generation rates (10^{-8}–10^{-7} Ms^{-1}), which are inaccessible at low temperatures for the known azoinitiators. By their activity in the generation of radicals in organic media the surfactants can be arranged in the following order, which indicates the essential role of counter ions in the catalytic action of CS:

CTAC ≈ TDTAC > CTAB ≈ CPB > DCDMAB > CTAHS

Surfactant	Cumene hydroperoxide, 37°C		Hydrogen peroxide, 37°C		Limonene hydroperoxide, 60 °C
	ϖ_i, $(M\cdot s)^{-1}$ Organics	ϖ_i, $(M\cdot s)^{-1}$ Water	ϖ_i, $(M\cdot s)^{-1}$ Organics	ϖ_i, $(M\cdot s)^{-1}$ Water	ϖ_i, $(M\cdot s)^{-1}$ Organics
CTAC	$2{,}1\cdot10^{-3}$	$3{,}7\cdot10^{-4}$	$0{,}67\cdot10^{-3}$	$0{,}14\cdot10^{-3}$	$27\cdot10^{-3}$
CTAB	$1{,}9\cdot10^{-3}$	$1{,}9\cdot10^{-4}$	$0{,}2\cdot10^{-3}$	$0{,}14\cdot10^{-3}$	$3{,}6\cdot10^{-3}$
CTAHS	$0{,}17\cdot10^{-3}$	$1{,}1\cdot10^{-4}$	≈0	≈0	$2{,}5\cdot10^{-3}$
DCDMAB	$1{,}5\cdot10^{-3}$	$2{,}8\cdot10^{-4}$	$2{,}1\cdot10^{-3}$	$0{,}37\cdot10^{-3}$	$3{,}6\cdot10^{-3}$
CPB	$1{,}9\cdot10^{-3}$	$3{,}3\cdot10^{-4}$	$0{,}2\cdot10^{-3}$	$0{,}14\cdot10^{-3}$	$3{,}6\cdot10^{-3}$
TDTAC	$2{,}1\cdot10^{-3}$	$3{,}7\cdot10^{-4}$	$0{,}47\cdot10^{-3}$	$0{,}13\cdot10^{-3}$	-
SDS	≈0	≈0	≈0	≈0	≈0
Lecithin	≈0	≈0	≈0	≈0	≈0

Table 8. Specific rates of radical initiation in the system: cationic surfactant + hydroperoxide in chlorbenzene and in water solution [47]

Along with hydroperoxide, water, and other polar oxidation products, catalytic and inhibiting components can be concentrated in mixed micelles {nLOOH·mS}. The combination of cationic surfactants with transition metal compounds known

as homogeneous catalysts of the hydrocarbon oxidation was found to demonstrate synergism, i.e., for the mixture of components the oxidation rate (R_Σ) exceeds the sum of the rates in the experiments with separately used components (R_{Me} and R_S): $\beta = R_\Sigma/(R_{Me} + R_S) > 1$. The ethylbenzene is oxidized selectively into acetophenon and water catalyzed with the combination of CTAB and cobalt acetylacetonate [53]. Under similar conditions limonene is oxidized with the primary formation of a carbonyl compound (carvon) [51].

Let us look on the mixed micelles of hydroperoxide and cationic surfactant once more. In mixed micelle, peroxide bond is localized in the interphase which has very strong intensity of electric field, about $5 \cdot 10^5$V/m. It affects peroxide bond, weakens it and facilitates decomposition into free radicals. Apparent activation energies of hydroperoxide decay decrease to 50-60 kJ/mol in mixed micelles from ~ 100 kJ/mol for thermal decay [51].

In the case of anionic surfactant the direction of electric field is different and decomposition into radicals is not facilitated. On the contrary, alkali metal alkyl sulfates [47,48] and alkyl phosphates [54,55] act as antioxidants to retard or completely suppress the oxidation process.

Nonionic surfactants form neutral micelles which have no electric field. May be, by that reason nonionic surfactants do not affect free radical formation in hydroperoxide decay, although they form mixed micelles {LOOH…S} with nonionic surfactant as well.

5.2. Phospholipid oxidation.

Phospholipids (PL) are natural surfactants, which are widely used in the production of food, drug, and cosmetics. PL are the basic lipid components of plasmatic cell membranes and membranes of subcellular organelles of animals, plants, and microorganisms. Phosphatidylcholines (1,2-diacyl-sn-glycero-3-phosphocholines, lecithins) are the most widely used; they are present in large amounts in myocardium, liver, kidneys, and egg yolk [56]. In lecithin molecules, anionic phosphate and cationic choline (tetraalkylammonium) groups are connected via a zwitterionic bond to form a neutral polar head. Hydrocarbon moiety represents residues of fatty acids, whose composition depends on the type of PLs (egg, soybean, fish, etc.). Lecithins have a zwitterionic structure in a wide pH range.

Unsaturated fatty acid residues of PL are readily oxidized with atmospheric oxygen as well as nonpolar unsaturated lipids. The primary products of PL oxidation are mainly isomeric hydroperoxides [9,12,57-59]. Lecithins are easily dissolved in organic solvents to yield compact reverse micelles [60,61]. In aqueous solutions, lecithin forms multilamellar liposomes or vesicles under the action of ultrasound dispersion [12,56,62]. Using the DLS method, it was found that, at egg lecithin concentrations 10–90 mg/mL, the size of micro-aggregates observed are equal to 5-6 nm in organic solvents and in water, liposomes are formed with a wide size distribution of 60–1000 nm.

PC oxidation in the presence of azoinitiators or transition metals occurs via free radical chain mechanism. The formation of micro-aggregates both in organic and water media results in a nonlinear dependence of the rate of oxygen absorption on substrate concentration (at constant initiation rate). The deviations from the linearity were observed at concentrations of egg lecithin above 5 mg/mL, corresponding to the formation of micro-aggregates. The rate increment caused by a further increase in the concentration markedly decreases [60,61]. It is possible that a partial shielding of active C–H bonds, which interact with peroxyl radicals from an initiator in solvent bulk results in a relative decrease in the oxidation rate.

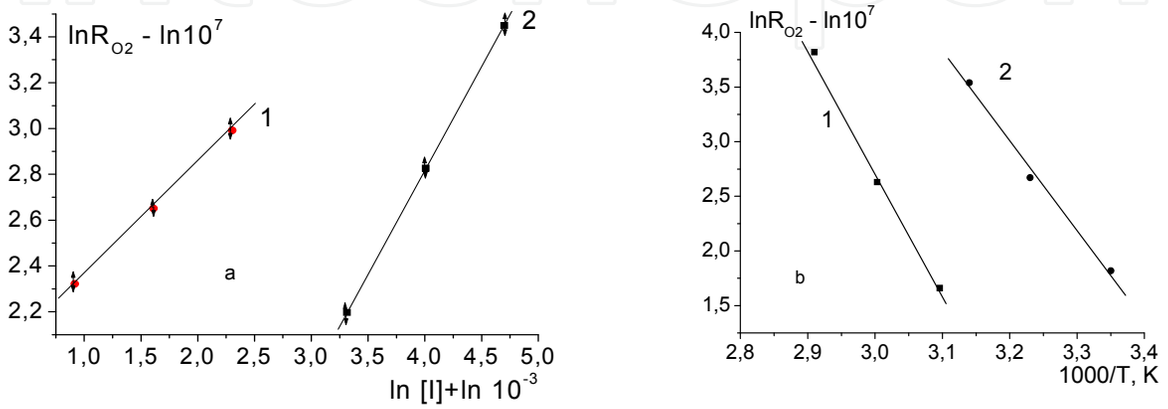


Figure 13. a) Dependences of lecithin (45mg/mL) oxidation rates on the initiator concentration in logarithmic coordinates: 1 – in n-decane solution, 60°C, I – azobisisobutyronitrile, AIBN; 2 – in water, 37°C, I – azodiiso-butyramidine-dihydrochloride (AAPH); b) Temperature dependence of the rate of lecithin (45mg/mL) oxidation plotted in Arrhenius coordinates: 1 – in chlorbenzene, [AIBN]= 5mM; 2 – in water, [AAPH]=55mM.

It was shown in [60,61] that the dependences of PC oxidation rates on initiation rates differ in organic and water solutions. In organic solvents, R_{O2} is proportional square root of R_i, whereas in water, $R_{O2} \sim R_i$. It can be seen from Fig.13a, where the dependences of egg lecithin oxidation rates on the corresponding initiator concentration (it means $R_{O2} - R_{IN}^n$, because $R_{IN} = k_i$[initiator]) are presented: in organics (chlorobenzene) n = 0,5 and n ≈ 1 in water media.

So, the rate of PL oxidation in an aqueous medium cannot be described by Eq. (1), common for lipid and hydrocarbon oxidation. Therefore, even in a narrow concentration range, it is unreasonable to compare the oxidizability of PL in water and an organic medium using parameter $a = k_p/(2k_t)^{0.5}$. Nevertheless, in many studies devoted to the oxidation of phospholipids in various media, Eq. (1) was applied to describe the rate of oxidation (absorption of oxygen [63-66] or accumulation of hydroperoxides [9,58,67]) and to determine the oxidizability of PLs or individual phosphatidylcholines [68]. The majority of these works was carried out at the physiological temperature (37°C). The measurements were performed in different ranges of the overall concentrations of PL and with different initiators and inhibitors used to determine the initiation rates; therefore, the conclusions were very different right up to the opposite ones.

According to [58,68] the oxidizability of PLs in aqueous dispersions is lower than that in organic solvents by an order of magnitude; it is higher in reverse micelles than in molecular alcohol solutions [58]. In [65,68], it was assumed that the micro-heterogeneity of PL solutions and the dispersity of colloidal solutions do not influence the oxidizability of unsaturated lipids in both aqueous and organic media.

A comparison of the experimentally measured rate of O_2 absorption during PL oxidation in aqueous solutions with the corresponding values obtained in an organic solvent at the same temperature, mass concentration of PL, and the initiation rate, which is governed by the contents of water- (AAPH) and oi-soluble (AIBN) azo-initiators, respectively, in the volumes of the solvents demonstrates the following [61]. At a temperature of 45°C, radical initiation rate of $22.5 \cdot 10^{-8}$ M/s, and PL concentration of 45 mg/mL, the rates of oxygen absorption in water and chlorobenzene are $3.5 \cdot 10^{-6}$ and $2.1 \cdot 10^{-6}$ M/s, respectively. A comparison suggests that, in the presence of a source of radicals, PL organized into multilamellar liposomes is ~1.5_fold faster oxidized in water than in the organic solution of reverse micelles. In order to explain this result, for micro-heterogeneous systems, one must introduce the concept of the effective (apparent) concentration of an oxidized substrate. In a system of multilamellar liposomes, the effective concentration of the oxidized substrate is higher than that in a system of reversed micelles occurring in an organic medium; therefore, a higher oxidation rate is observed at the same temperature and the rate of radical initiation. The rates of PL oxidation both in organics and in water, initiated by corresponding initiator, increase with temperature according to the Arrhenius equation (Fig.10b). The effective activation energy of AAPH-initiated PL oxidation in an aqueous solution (74 kJ/mol) is lower than the activation energy of AAPH decomposition (112 kJ/mol). Hence, a radical chain mechanism of PL oxidation in water is more complex than described above mechanism of model oil oxidation (Table 1). In a micro-heterogeneous medium, in addition to individual radicals and molecules, reagents that are included into microaggregates (liposomes) and characterized by reactivity different from that of molecular_dispersed particles in corresponding reactions are involved in the stages of chain initiation, propagation, and termination. Cross-disproportionation reactions of different radicals occur to result in the imitation of a linear chain termination.

α-Tocopherol is well known the most effective lipid antioxidant [1-3]. Lecithin liposome oxidation in the presence of α-tocopherol demonstrates that marked induction periods may be observed when α-tocopherol is added inside liposome during preparation. The incomplete suppression of O_2 absorption by α-tocopherol may be indicative of the PC oxidation inside of liposome without migration of peroxyl radicals into the bulk solvent.

It turns out that catecholamines dopamine, adrenaline and noradrenaline are much more strong and effective inhibitors for PC oxidation than α-tocopherol (Compare Fig.14 and Fig.15). Evidently, the positive charge of catecholamines at neutral pH facilitates their adsorption and protective action on the surface of negative charged liposomes [61].

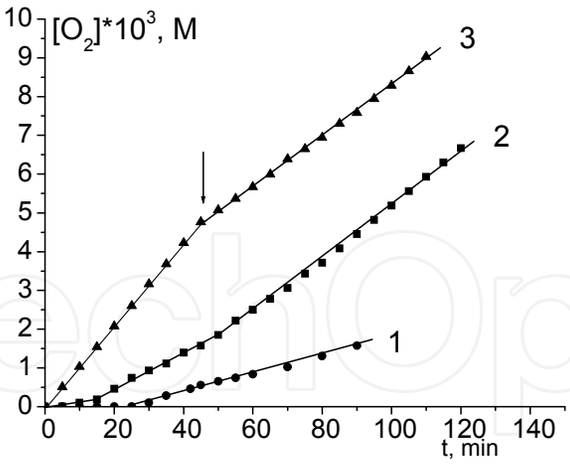


Figure 14. Kinetic curves for O_2 absorption during AAPH- initiated (55 mM) oxidation of PL (45 mg/ml) in water at 37°C in the presence of (1) 0.33 and (2) 0.05 mM α-tocopherol incorporated upon preparation of liposomes and (3)0.05 mM α-tocopherol introduced directly into solution

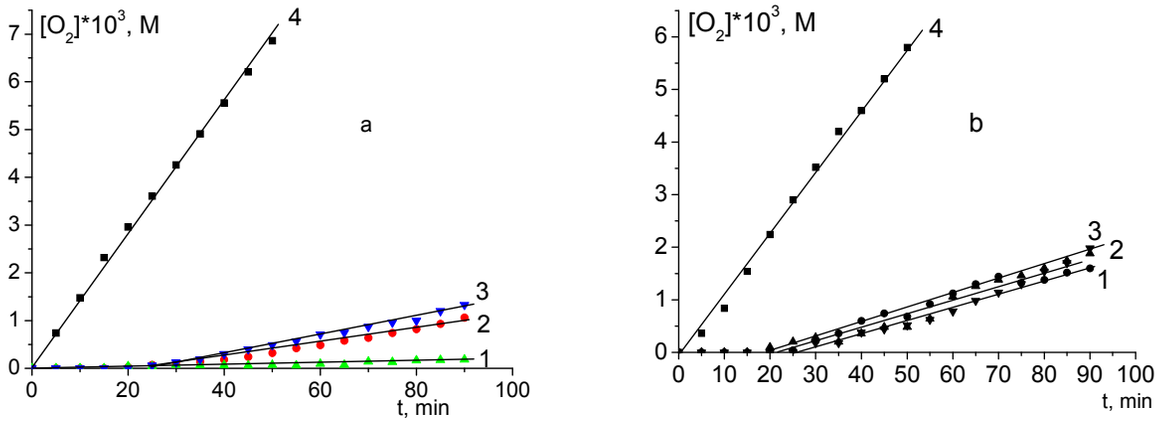


Figure 15. Effect of 0.1 mM (1) adrenalin, (2) dopamine, (3) noradrenalin on AAPH-initiated (55 mM) oxidation of PL (45 mg/ml) at 37°C in (a) water and (b) in phosphate buffer with pH 7.4; (4) no additives.

It must be noted that in the phosphate buffer, induction periods τ are nearly equal for all of the catecholamines (Fig.15b), while, in an aqueous solution, adrenalin provides a longer inhibition of the oxidation than dopamine and noradrenalin do (Fig.15a). The analysis of the ratios between the rates of radical initiation and the durations of the induction periods testified that, for all catecholamines in the buffer solution, the stoichiometry of inhibition, which is numerically equal to the number of radicals corresponding to one acceptor molecule, is $n = (R_{IN} \cdot \tau)/[CA]_0 = 2$, which is characteristic of catechols. In an aqueous and a physiological solution (0.9% NaCl), dopamine and noradrenalin exhibit n= 2, while for adrenaline, n= 4. Moreover, the adrenalin-containing mixture acquires a pink color in water.

Figure 16a illustrates variations in the optical absorption spectra of adrenalin solutions in water and a physiological solution (0.9% NaCl) during its free-radical oxidation initiated by AAPH. It can be seen that the oxidation results in the formation of a colored product with an

absorption maximum at 480 nm. In the phosphate buffer of pH 7.4, adrenalin also undergoes transformations (Fig. 16b); however, they yield no colored product. The spectral characteristics of the pink product ($\varepsilon = 4.02 \times 10^3$ M^{-1} cm^{-1} at 480 nm) correspond to adrenochrome (3-hydroxy-1-methyl-2,3-dihydro-1H-indole-5,6-dion), which is formed via the abstraction of four hydrogen atoms from adrenaline [68].

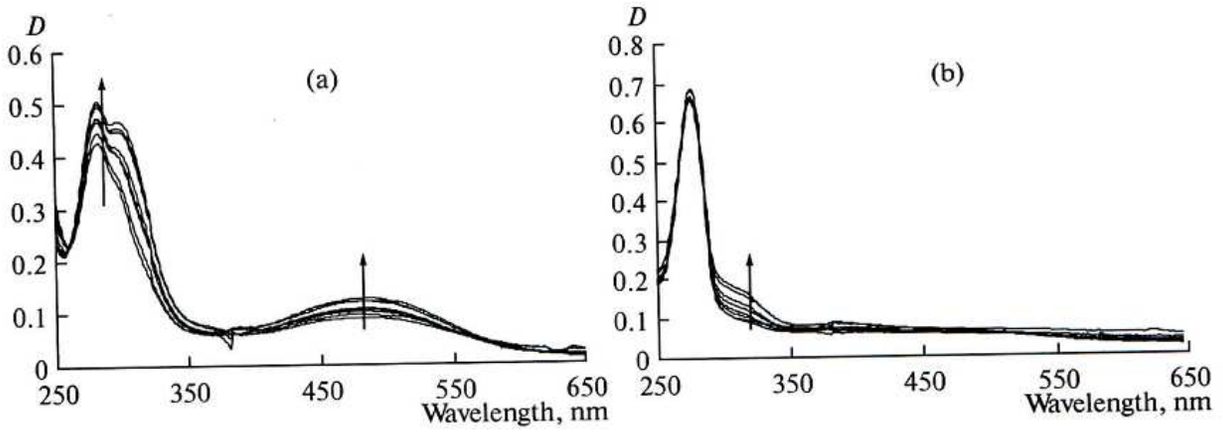


Figure 16. Variations in UV spectrum of 0.1 mM adrenalin solution in the process of its oxidation at 37°C (a) in water and 0.9% NaCl solution and (b) in phosphate buffer with pH 7.2.

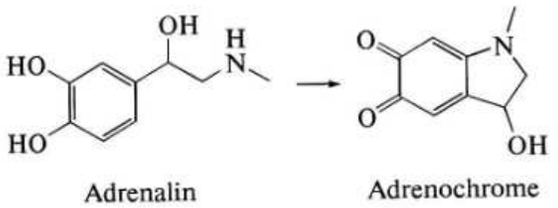


Figure 17. Structures of adrenalin and adrenochrome

It is interesting that, in an aqueous solution, under the conditions of AAPH-initiated free-radical oxidation, adrenalin is quantitatively transformed into adrenochrome. However, in the phosphate buffer solution adrenochrome is not formed.

6. Concluding remarks

Radical scavenging activity towards DPPH radical gives information only about the H-donating capacity of the studied compounds and some preliminary information for their possibility to be used as antioxidants. Antioxidant activity is capacity of the compound to shorten the oxidation chain length as a result of its reaction with peroxyl radicals. For that reason we mean as antioxidant activity the chain-breaking activity of the compounds. This comparable study showed a good correlation between experimental antioxidant activity of compounds under study and their predictable activity by using TLC DPPH radical test.

It has been demonstrated that phenolic compounds with catecholic moiety are the most powerful scavengers of free radicals and they may be used as effective chain-breaking antioxidants. The highest antiradical and antioxidant activity of phenolic antioxidants with

catecholic moiety is explained by possible mechanism of homo-disproportionation of their semiquinone radicals formed.

Thus regeneration of the antioxidant molecule during the oxidation process is possible. It has been found for the first time that only substitution in the aromatic nucleus of the studied bis-coumarins and xanthenes-lignans is responsible for their antioxidant activity.

It must be noted that antioxidants' activity depends significantly not only on their structural characteristics, but also on the properties of the substrate being oxidized and the experimental conditions applied. Structural characteristics of the complex system: oxidizing substrate - antioxidant must be considered. On the basis of this comparable analysis, the most effective individual antioxidants and binary mixtures were proposed for highest and optimal lipid oxidation stability.

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