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MECHANISM OF INHIBITION OF CUMENE OXIDATION THE EXTRACT OF FLAX SEEDS

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Antioxidant property of the ethylacetate extract of flax seeds was studied by the example of model reaction of the initiated oxidation of cumene. It was established that extract of flax seeds exhibits antioxidant property at cumene oxidation. However, in contrast to classical inhibitors (phenols, aromatic amines, etc.) kinetic curves of oxygen absorption in the presence of flax seeds extract were not characterized by an induction period. At that, in parallel with increasing in content of extract in the reaction mixture the velocity of cumene oxidation decreased on certain degree and became independent of its concentration. A mechanism was suggested to describe the effect of flax seeds extract on the kinetics of cumene oxidation (RH). $И \rightarrow RO_2'(V_i)$

$$RO_2' + \ln H \xrightarrow{K} RO_2 ... H \ln$$

$$RO_2' + RH \xrightarrow{K_2} ROOH + R'$$

$$RO_2' ... H \ln + RH \xrightarrow{K_{\cdot 2}} ROOH + \ln H + R'$$

$$\left.\begin{array}{r} RO_2' + RO_2' \xrightarrow{K_6} \\ RO\cdot_2 ... H \ln + RO\cdot_2 \xrightarrow{K_7} \\ RO\cdot_2 ... H \ln \xrightarrow{K_{\cdot 7}} \end{array}\right\} \text{Molecular products}$$

According to the suggested mechanism the limit velocity of cumene oxidation is described by the equation $V_\infty = \frac{[RH]V_i}{2k_7'}$ *, and at low concentrations when not all* RO_2' *radicals are in associates (* $RO_2 ... H \ln$*), then:*

$$\frac{V_0}{V} - \frac{V}{V_0} = \frac{2k_7' K[\ln H]_0}{(k_6 V_i)^{\frac{1}{2}}}$$

where V_o and V are the velocities of cumene oxidation with and without the extract, respectively.

Temperature dependences of $\frac{k_2'}{k_7'}$ *and* $k_7' K$ *, characterizing antioxidant activity of the extract of flax seeds were determined. It was revealed that*

$$\frac{k_2'}{k_7'} = 3{,}38 \cdot 10^7 exp[-(10550 \pm 50)/RT]$$

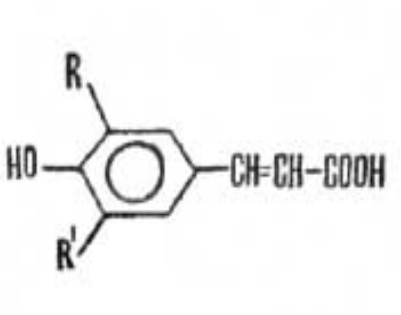
$$k_7'[K] = 1{,}69 \cdot 10^{11} \exp[-(13850 \pm 50)/RT$$

Key words: flax, antioxidant, inhibitor, oxidation of cumene , peroxide radical**.**

Introduction

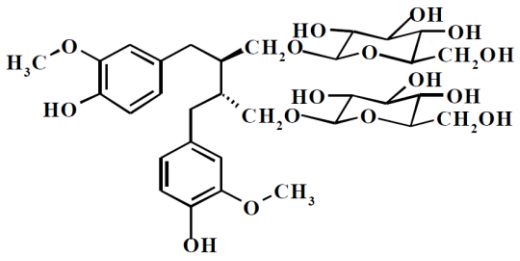
Flax oil has used both in cooking and in folk and scientific medicine since ancient time to cure various diseases. Its beneficial actions are due to the rich content of biologically active substances. In particular, the composition of flax seed of canadian varieties on a dry matter basis the following: fat component is 41%, proteins -21%, cellulose-28%, aromatic acids, lignin, phenolic compounds-6%, ash-4% [1]. It should be noted that the composition of flaxseed (as well as the composition of the essential oil of any medicinal plants) varies with varieties, environment, seasonal growing conditions and methods of processing flax [2].

Of phenolic compounds in flaxseed encountered phenolic acids: ferulic, Trans-sinova, Trans-coumaric, Ttans-caffeic, from 7.9 to 10.3 mg/g [3], and lignans –mostly diglycoside secoisolariciresinol (SDG)-from 13.6 to 32.1 mg/g [4].



R=R'=H, coumaric acid,
R=H, R'=OH, coffee acid,
R=H, R'=OCH_3 ferulic acid,,
R=R'=OCH_3 sinova acid

Established [5,6] that the physiological effects of phenolic compounds in plants is the regulation of growth and reproduction in plant protection from the harmful effects of UV radiation, infection of plants by fungi, to control the actions of other biologically active compounds. It is indicated [7,8] that the lignans of flax seeds can be used for therapeutic purposes to inhibit and stop the growth of tumor cells as antiallergen in the treatment of atherosclerosis and coronary heart failure. In addition, these phenolic compounds should have strong antioxidant properties. To test this, in this thesis we have been studied the ethyl acetate antioxidant activity of the extract of flax seeds on example of model reaction of cumene oxidation.



SDG

The experimental part

The flax seeds purchased in the supermarket in Yerevan . The extract was obtained as follows: flax seeds are dried in a drying cabinet at a temperature of 318K to constant weight, then rubbed in a ceramic mortar, the resulting mixture was added ethyl acetate ratio of 1:20 (1G of a mixture of 20ml of solvent). As a result got extract in the form of a clear slightly yellow liquid with a density of 0,8520 g/ml and an optical density D20=1,4850. To study the antioxidant action of the extract of flax seeds (EFS) in the radical-chain oxidation processes in the model system was chosen of the initiated liquid phase oxidation of cumene, for which the mechanism of all elementary steps are well studied [9]. Initiated azodiisobutyronitrile (AIBN) oxidation of cumene was studied by varying the content of the EFS and the concentration of AIBN in the environment of chlorobenzene in the

temperature range of 328-348K. The concentration of cumene in all experiments was of 2.87 mol/l, the volume of the reaction mixture 5ml.

The kinetic process of the oxidation of cumene was observed by gasvolumetric method , measuring the amount of absorbed oxygen at a given temperature in an oxygen atmosphere on the setup described in the paper [9].

The study of the oxidation process was carried out in the kinetic region where the rate of oxygen uptake did not depend on the stirring speed of the reaction mixture. Used reagents, cumene, chlorobenzene, AIBN, ethyl acetate were purified according to the method described in the paper [10].

Results and discussion

Figure 1 presents typical kinetic curves of oxygen absorption of the oxidized cumene respectively, in the absence of the studied extract (D. 1) and in the presence of extracts from the seeds of flax (curves 2 and 3). From the kinetic curves it is seen that in the presence of the investigated extracts, unlike previously, we investigated extracts of several medicinal plants [11-12], oxygen uptake proceeds without induction periods, i.e. extract flax seed acts as a moderator of the oxidation process. Moreover, with increasing amounts of the dissolved extract in the reaction mixture the rate of oxygen uptake, decreasing, tends to a constant value (Fig.2) and further depends on concentration of ESL. A similar phenomenon was detected at different temperatures. The results of these study are given in the table.

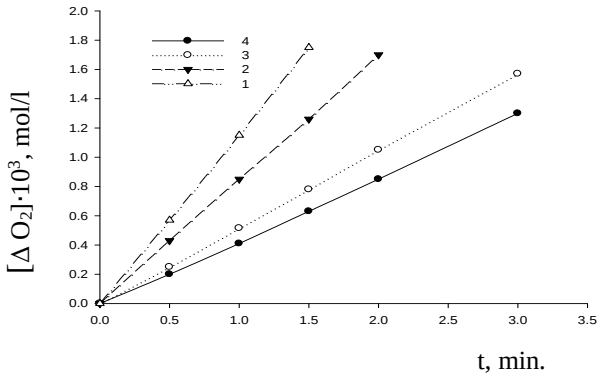


Figure 1. Kinetic curves of O2 absorption, oxidizing cumene in the absence (1) and in the presence of 2 (2) 4,8 (3) and 12mg (4) EFS. Vi=0,78·10-7 mol/l·s 339 K.

For experimentally discovered facts it is possible to give the following explanation. The oxidation of organic matter by peroxide radicals react with antioxidants (in our case of phenols) taking the *H*-atom from the *O-H*. In the past century it has been proven that peroxide radicals form a hydrogen bond with a hydroxyl-bearing compounds [13,14].

$$RO_2^{\cdot} + HOR' \Leftrightarrow RO_2^{\cdot} \ldots HOR'$$

Since EFS is contained in a sufficient amount of phenolic compounds (see introduction), they also like water and alcohols can form associates with the peroxide radicals of the type *PhOH...O_2^.R*. As the peroxide radical removes *H-atom* from the same context, therefore, the reaction ⟦*RO*⟧ *_2^. InH* can be presented as follows

$$RO_2^{\cdot} + InH \Leftrightarrow RO_2^{\cdot} \ldots HIn \xrightarrow{k_7'} ROOH + In^{\cdot}$$

$$In^{\cdot} + RO_2^{\cdot} \ldots HIn \xrightarrow{k_8} \text{molecular products}$$

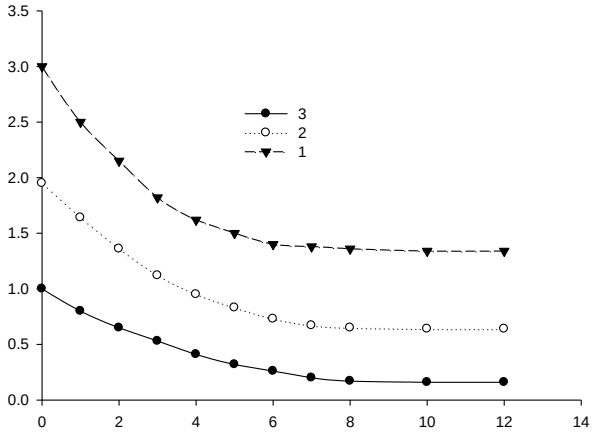


Figure 2 . The dependence of the rate of oxidation of cumene from the content содержания EFS.

1) V_i=1,25 · 10^{-7}M·c^{-1}; 348K, 2) V_i=7,83 · 10^{-8}M·c^{-1}; 339K, 3) V_i=3,44 · 10^{-8}M·c^{-1}; 328K

This mechanism leads to the following conclusions.

1. It is known that the peroxide radical, linked by hydrogen bond, can continue the chain [13]. Obviously, the same property must have a radical *〖RO〗_2^'HIn...*

$$RO_2^{\cdot} \ldots HIn + RH \xrightarrow{k_2^{\cdot}} ROOH + InH + R^{\cdot}$$

At sufficiently high concentrations of the inhibitor (in our case, EFS), when all *〖RO〗_2^.* radicals are in the form of associate *〖RO〗_2^....*HIn, there should be a limit on the oxidation rate independent of the concentration of the inhibitor. In these conditions the rate of chain oxidation equal

$$V_\infty = k_2^{'}[RH][RO_2^{\cdot} \ldots HIn] = k_2^{'}[RH]V_i/2k_7^{'} \qquad (1)$$

2. At relatively low concentrations of the inhibitor (EFS) when the oxidation rate depends on its concentration, (*V>V_∞)* an open circuit can occur in the reaction *〖RO〗_2^....HIn (→⊥k_7^') ROOH 〖In〗^.*

and the reactions *〖〖RO〗_2^. RO〗_2^....HIn(→⊥k_7 ROOH ROOIn)*

$$RO_2^{\cdot} + RO_2^{\cdot} \xrightarrow{k_6} \text{molecular products}$$

Such breakage of the chains leads to the following dependence of the oxidation rate on the concentration of the inhibitor (EFS)

$$V = k_2[RH][RO_2^{\cdot}] \qquad (2)$$

Using the condition of stationarity for $RO2.$ radicals, get

$$V_i = k_6[RO_2^{\cdot}]^2 + 2k_7'[RO_2^{\cdot} \ldots HIn] + 2k_7[RO_2^{\cdot}][RO_2^{\cdot} \ldots HIn] \qquad (3)$$

Hence, determining the concentration *〖RO〗_2^* radicals and putting in equation (2), given that

[〖RO〗_2^....HIn]=K[InH][〖RO〗_2^.], get

$$V = k_2[RH]\frac{k_7'K[InH]}{k_6+2k_7K[InH]}\left\{\left(1+\frac{v_i(k_6+2k_7'K[InH])}{(k_2'K[InH]^2)}\right)^{1/2}-1\right\} \qquad (4)$$

given that the rate of oxidation of cumene in the absence of inhibitor is described by the equation

$$V_0 = \frac{k_2}{\sqrt{k_6}}[RH]\sqrt{V_i} \quad (5)$$

and that in the initial moment of time [*InH]= 〖[InH]〗_0*, get

$$\frac{V_0}{V} - \frac{V}{V_0} = 2k_7' K[InH]_0/(k_6 V_i)^{1/2} \quad (6)$$

Where *V*_i is the speed of initiation, *[InH]_0* is the initial concentration of inhibitor and antioxidants in the studied extracts, *K* is a constant balance *〖RO〗_2^. InH(⇔⊣.) ROOH 〖In〗^'.*

The concentration of antioxidant substances determined on the assumption that in the extract of flax seed contains about 13.7% lignin [4].

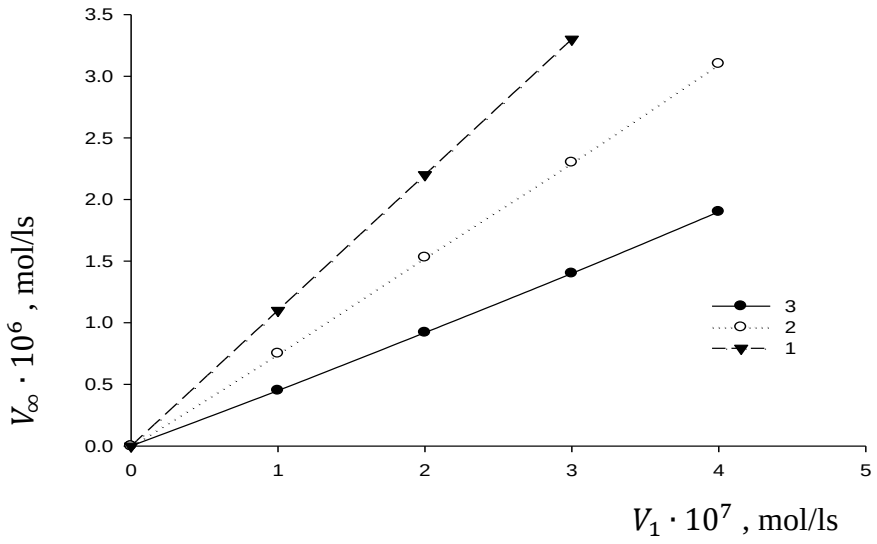


Figure 3. The dependence of the limiting oxidation rate of 2.87 M cumene speed of initiation. MELs =12mg; T=348 (1); 339 (2) и 328 К (3).

From figure 3 it follows that the detected speed limit oxidation of the dome described by the equation (1). Where calculated temperature dependence of the ratio of the rate constants of the reaction *k_2^'/k_7^'* in Arrenius coordinates. It is obtained that

$$\frac{k_2'}{k_7'} = 3{,}38 \cdot 10^7 exp[-(10550 \pm 50)/RT] \quad (7)$$

At relatively low concentrations of EFS ($m_{EFS} < 2$мг), when chains are torn as linear and quadratic, i.e. in terms of $V > V_\infty$ the experimental data are described (Fig. 4) by equation (6). Using the results of table and figure. 4, given the fact that for cumene *k_6=4,74· 〖10〗^5 e^(-1800/RT)* [15], the determined temperature dependence of the product of the constants *k_7^' K.*

Table 1.

Data for the oxidation of 2.87 mol/l of cumene in the presence of EFS

$T; K$	$m_{ЭСЛ}$, мг	$[InH] \cdot 10^4$, моль/л	$V_i \cdot 10^7$, моль/л·с	$V_{O_2} \cdot 10^6$, моль/л·с	$\frac{V_0}{V} - \frac{V}{V_0}$	k_2'/k_7'	$k_7' \cdot K \cdot 10^{-2}$
348	0	0	1,25	3,00	.	.	.
348	0,5	0,219	1,25	2,67	0,22	-	3,34
348	1,0	0,437	1,25	2,41	0,44	-	3,34
348	1,5	0,656	1,25	2,22	0,61	-	3,40
348	2,0	0,875	1,25	1,99	0,83	-	3,16
348	4,0	1,749	1,25	1,75	1,13	-	-
348	8,0	3,498	1,25	1,60	1,34	-	-
348	12,0	5,247	1,25	1,40	1,50	8,10	-
348	12,0	5,247	0,50	0,60	1,15	8,36	-
348	12,0	5,247	2,50	2,80	0,70	7,80	-
339	0	0	0,783	2,00	-	-	-
339	0,5	0,219	0,783	1,85	0,16	-	1,89
339	1,0	0,437	0,783	1,69	0,33	-	1,90
339	1,5	0,656	0,783	1,56	0,50	-	1,91
339	2,0	0,875	0,783	1,40	0,73	-	2,12
339	4	1,749	0,783	1,00	1,50	-	-
339	8	3,498	0,783	0,72	2,50	-	-
339	12	5,247	0,783	0,60	3,00	5,38	-
339	12	5,247	1,50	1,20	1,92	5,57	-
339	12	5,247	2,45	1,85	1,39	5,26	-
339	12	5,247	3,10	2,20	3,00	5,00	-
339	16	7,996	0,78	0,60	3,00	5,34	-
328	0	0	0,344	1,00	-	-	-
328	0,5	0,219	0,344	0,94	0,125	-	0,91
328	1,0	0,437	0,344	0,88	0,25	-	0,90
328	1,5	0,656	0,344	0,83	0,37	-	0,90
328	2,0	0,875	0,344	0,78	0,50	-	0,91
328	4,0	1,749	0,344	0,42	1,96	-	-
328	8,0	3,498	0,344	0,15	6,50	3,13	-
328	12,0	5,247	0,344	0,15	6,50	3,18	-
328	12,0	5,247	1,680	0,80	2,41	3,32	-
328	12,0	5,247	3,000	1,35	1,73	3,14	-
328	12,0	5,247	4,000	1,85	1,30	3,22	-

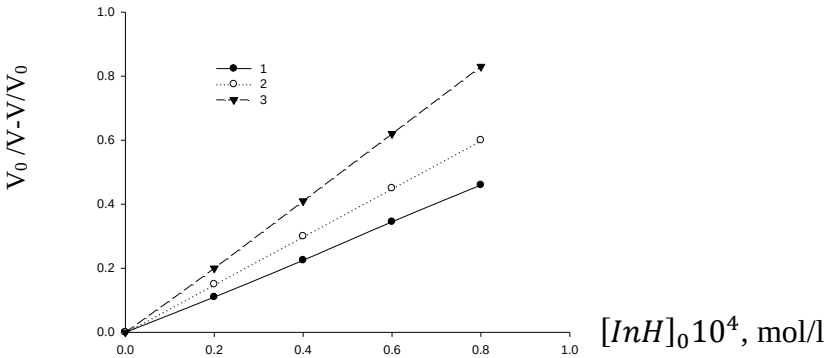


Figure 4. The dependence of the parameter V0/V-V/V0 on the concentration of inhibitors contained in EFS.

$$k_7'[K] = 1{,}69 \cdot 10^{11}\exp[-(13850 \pm 50)/RT \quad (8)$$

1) V_i=1,25 · 10^{-7} M·c^{-1}; 348K, 2) V_i=7,83 · 10^{-8} M·c^{-1}; 348 K, 3) V_i=3,44 · 10^{-8} M·c^{-1}; 348K

Conclusion

On the basis of kinetic data of oxidation of cumene in the presence of the extract of flax seeds proposed mechanism for the reaction of peroxide radicals with inhibitors, including preliminary education in between associates through hydrogen bond, values of *V_(∞) , V_0/V-V/V_0 and k_(6*) the calculated constants *(k_2^')/(k_7^') and k_7^' [K]*.

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Կումոլի հարուցված օքսիդացման մոդելային ռեակցիայի օրինակի վրա հետազոտվել է կտավատի սերմերի էթիլացետատային էքստրակտի անտիօքսիդանտ հատկությունները: Հաստատվել է, որ կումոլի օքսիդացման ժամանակ, կտավատի սերմերի էքստրակտը անտիօքսիդանտ հատկություններ է ցուցաբերում: Ի տարբերություն դասական ինհիբիտորների (ֆենոլներ, արոմատիկ ամիններ և այլն), կտավատի սերմերի էքստրակտի ներկայությամբ թթվածնի կլանման կինետիկական կորերը անցնում են առանց ինդուկցիոն ժամանակահատվածների: Ընդ որում, տրված հարուցման արագության դեպքում ռեակցիոն խառնուրդում էքստրակտի պարունակությունը ավելացնելիս, կումոլի օքսիդացման արագությունը դանդաղում է մինչև որոշակի աստիճան և կախված չէ նրա կոնցենտրացիաըից:

Առաջարկված է, կումոլի օքսիդացման կինետիկայի վրա կտավատի սերմերի էքստրակտի աղդեցության մեխանիզմը (RH). $И \rightarrow RO_2^{\cdot}\ (V_i)$

$$RO_2^{\cdot} + InH \overset{K}{\rightarrow} RO_2^{\cdot} \ldots HIn$$
$$RO_2' + RH \overset{k_2}{\rightarrow} ROOH + R'$$
$$RO_2^{\cdot} \ldots HIn + RH \overset{k_2}{\rightarrow} ROOH + InH + R'$$
$$\left.\begin{array}{c} RO_2' + RO_2' \overset{k_6}{\rightarrow} \\ RO_2^{\cdot} \ldots HIn + RO_2^{\cdot} \overset{k_7}{\rightarrow} \\ RO_2^{\cdot} \ldots HIn \overset{k_7'}{\rightarrow} \end{array}\right\} \text{Մոլեկ. պրոդուկտներ}$$

Համաձայն առաջարկված սխեմայի կումոլի օքսիդացման առավելագույն արագությունը (V_∞) նկարագրվում է $V_\infty = [RH]V_i/2k_7'$ հավասարմամբ, իսկ ցածր կոնցենտրացիաների դեպքում, երբ ոչ բոլոր RO_2' ռադիկալներն են գտնվում ասոցիատներում ($RO_2^{\cdot} \dots HIn$)

$$\frac{V_0}{V} - \frac{V}{V_0} = 2k_7'K[InH]_0/(k_6V_i)^{1/2}$$

Որտեղ, V_0 և V –կումոլի օքսիդացման արագություններն են (էքստրակտի առկայությամբ և բացակայությամբ):

Որոշված են էքստրակտի անտիօքսիդանտ ակտիվությունները բնութագրող ջերմաստիճանային կախվածությունները $\frac{k_2'}{k_7'}$ և k_7'K : Ստացված է, որ $\frac{k_2'}{k_7'} = 3{,}38 \cdot 10^7 exp[-(10550 \pm 50)/RT]$, $k_7'[K] = 1{,}69 \cdot 10^{11}\exp[-(13850 \pm 50)/RT$

Բանալի բառեր: կտավատ, անտիօքսիդանտ, ինհիբիտոր, կումոլի օքսիդացում, պերօքսիդային ռադիկալ:

МЕХАНИЗМ ИНГИБИРОВАНИЯ ОКИСЛЕНИЯ КУМОЛА ЭКСТРАКТОМ СЕМЯН ЛЬНА

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На примере модельной реакции инициированного окисления кумола исследовано антиоксидантное свойство этилацетатного экстракта семян льна. Установлено, что экстракт семян льна проявляет антиоксидантное свойство при окисление кумола. Однако, в отличие от классических ингибиторов (фенолы, ароматические амиы и др.), кинетические кривые поглощения кислорода в присутствии экстракта семян льна проходят без периодов индукции. Причем, с увеличением содержания экстракта в реакционной смеси, при данной скорости инициирования, скорость окисления кумола замедляется до определенной степени и не зависит от его концентрации. Предложен механизм действия экстракта семян льна на кинетику окисления кумола (RH). И → $RO_2^{\cdot}$ (V_i).

$$RO_2^{\cdot} + InH \xrightarrow{K} RO_2^{\cdot} \dots HIn$$

$$RO_2' + RH \xrightarrow{k_2} ROOH + R'$$

$$RO_2^{\cdot} \dots HIn + RH \xrightarrow{k_2} ROOH + InH + R'$$

$$\left.\begin{array}{l} RO_2' + RO_2' \xrightarrow{k_6} \\ RO_2^{\cdot} \dots HIn + RO_2^{\cdot} \xrightarrow{k_7} \\ RO_2^{\cdot} \dots HIn \xrightarrow{k_7'} \end{array}\right\} \text{Молек. продукты}$$

Согласно предложенной схеме предельная скорость окисления кумола (V_∞) описывается уравнением $V_\infty = [RH]V_i/2k_7'$, а при низких концентрациях, когда не все RO_2' радикалы находятся в ассоциатах ($RO_2^{\cdot} \dots HIn$).

$$\frac{V_0}{V} - \frac{V}{V_0} = 2k_7'K[InH]_0/(k_6V_i)^{1/2}$$

где, V_0 и V –скорости окисления кумола, соответственно, в отсутствие и в присутствие экстракта.

Определены температурные зависимости $\frac{k_2'}{k_7'}$ и k_7'К, характеризующие антиоксидантные активности экстракта семян льна. Получено, что $\frac{k_2'}{k_7'} = 3{,}38 \cdot 10^7 exp[-(10550 \pm 50)/RT]$, $k_7'[K] = 1{,}69 \cdot 10^{11}\exp[-(13850 \pm 50)/RT$

Ключевые слова: лен, антиоксидант, ингибитор, окисление кумола, пероксидный радикал.