Antioxidant Effect of Some Flavonoids on Organic Substrate

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This investigation concerns the evaluation of the protection activity of natural antioxidants. Ethanol extracts form dry five plants (Salvia Officinalis, Rosmarinus Officinalis, Rhus Typhia, Origanum Vulgaris and Ocimum Basilicum) were prepared at room temperature. The types of flavonoids acting as antioxidant compounds were identified for establishing the structural correlation between functional substituents and protection efficiency. The isothermal chemiluminescence determinations allowed the calculation of kinetic parameters of stabilizing process. The mechanism steps of stabilization are proposed.

Keywords: natural antioxidants, flavonoids, chemiluminescence

It is well known that in the last years the general trend of inclusion of more and more natural additives or similar compounds in different products was noticed due to their well tolerated activity by the human body.

The addition of any new stabilizing compound to polymers must respect the requirements of ecology and industrial toxicology. Materials containing antioxidants have to be safely in contact with the packaged products and not hazardous for people health [1-6].

A large number of papers emphasized that different plants such as Salvia Officinalis, Rosmarinus Officinalis, Rhus Typhia, Origanum Vulgaris and Ocimum Basilicum are presenting a great applicative interest due to their considerable antioxidant properties in comparison with other plants. It was shown that different flavonoids are responsible of this antioxidant effect (carnosic acid, p-cumaric acid, chlorogenic acid, ferulic acid, rosmanol, carnosol etc.).

Experimental part

During the developed experiments different plant extracts were obtained using the cold extraction method (dry plant:ethanol = 1:10, for 5 days at room temperature). After drying under vacuum, the solid residuum was collected.

Paraffin was used such as oxidation substrate, being intimately blended with each natural extract previously obtained (0.25% w/w). The components were stirred by damping with trichloroethylene.

The chemiluminescence (CL), IR and UV-VIS spectroscopy were used as investigation techniques. IR and UV-VIS spectra were registered with a FTIR JASCO 4000 and JASCO V570 spectrometers. The isothermal chemiluminescence emission of the samples (153°C, in air presence) was registered with an Oxiluminograph OL-94 [7, 8]. The chemiluminescence curves were recorded from where kinetic parameters of thermal oxidation were calculated. For comparison, one commercial available antioxidant produced by synthesis (TOPANOL-OC) was also tested under similar conditions.

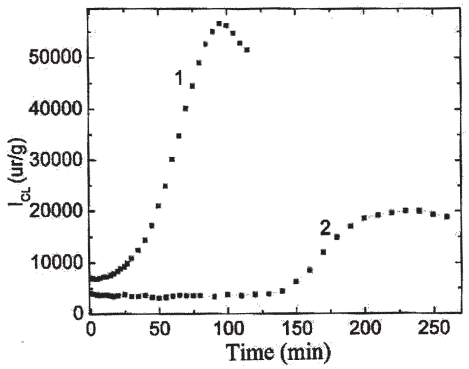

Fig.1. CL curves (153°C, air) of unstabilized (1) and stabilized (0,25% w/w TOPANOL-OC) (2) paraffin

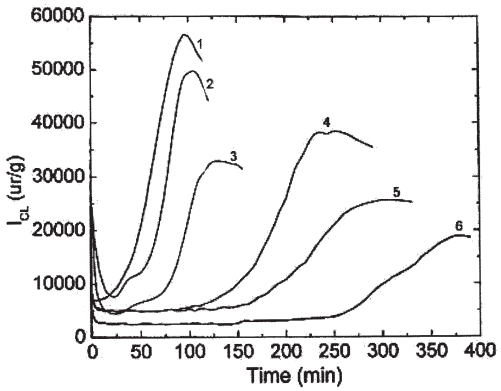

Fig. 2. CL curves (153°C, air) paraffin additivated (0,25% w/w) with plant extracts from Lamiaceae Family 1 – blank; 2 – Ocimum Basilicum; 3 – Origanum Vulgaris; 4 – Rhus Typhia; 5 – Rosmarinus Officinalis; 6 – Salvia Officinalis

Plant Extract	Natural antioxidant compound
Salvia Officinalis	Cafeic acid, Ursolic acid, Oleanolic acid, Chlorogenic acid
Rosmarinus Officinalis	Carnosic acid, Cafeic acid, Rosmarinic acid, Apigenin and Luteolin
Origanum Vulgaris	Rosmarinic acid, Cafeic acid, Apigenin, A and C Vitamins
Ocimum Basilicum	Cafeic acid, p-Cumaric acid, Quercitin and Rutin

Table 1
NATURAL COMPOUNDS WITH ANTIOXIDANT ACTIVITY

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Table 2

KINETIC OXIDATION PARAMETERS (153°C, AIR) OF PARAFFIN ADDITIVATED (0,25% W/W) WITH PLANT EXTRACTS FROM LAMIACEAE FAMILY AND TOPANOL-OC

Compound	t_i (min)	$t_{1/2}$ (min)	v_{ox}^{max} (u.r./g·min)	I_{max} (u.r./g)	t_{max} (min)
blank	22	56	958	56634	95
Salvia Officinalis	242	302	113	18937	375
Rosmarinus Officinalis	163	220	277	25636	300
Rhus Typhia	143	184	452	38398	235
Origanum Vulgaris	70	90	769	32965	130
Ocimum Basilicum	39	79	465	27525	130
TOPANOL-OC	133	164	285	20100	230

Results and discussions

The main compounds with antioxidant activity contained in the studied plant extracts are presented in table 1.

The kinetic parameters (the oxidation induction period, the attending of half maximum CL intensity period, the rate of oxidation and maximum CL intensity) were calculated from the registered CL curves.

Figure 1 presents the CL curves obtained for unstabilized and stabilized (0.25% w/w TOPANOL-OC) organic substrate (paraffin). Figure 2 illustrates the comparative CL curves of paraffin additivated with the plant extracts. The kinetic parameters resulted from these CL curves are emphasized in table 2. As it can be observed from this table, the Salvia Officinalis, Rosmarinus Officinalis and Rhus Typhia extracts lead to higher oxidation induction periods and lower oxidation rates than Origanum Vulgaris and Ocimum Basilicum. It also could be noticed the great antioxidant activity of Salvia Officinalis extract which present an eight time decreasing of the oxidation rate relative to the unstabilized substrate.

Comparative with TOPANOL-OC, the plant extracts present the following relative activity: 1.982 for Slavia Officinalis, 1.270 for Rosmarinus Officinalis, 1.090 for Rhus Typhia, 0.432 Origanum Vulgaris and 0.153 Ocimum Basilicum. In the same time, this extracts lead to fractional values of the relative oxidation rate. This proves that the antioxidant activity of these extracts is continuing not just in the oxidation induction period but also after the start of the oxidation process.

The remarkable antioxidant activity of these extracts could be explained by the presence of some natural polyphenols with a great efficiency in the free radicals scavenger process. The responsible compounds for this activity are: cafeic acid, chlorogenic acid, p-cumaric acid, ferulic acid, rosmarinic acid and salvianolic acid (fig.3).

The antioxidant activity is increasing with the active components concentration in the studied extracts. Figure 4 illustrates an example of the oxidation induction period dependence with the extract concentration.

The activation energy of the oxidation process was calculated from the CL curves registered to three different temperatures (156, 163, 178°C). Figure 5 illustrates CL curves registered to these temperatures for paraffin additivated with Ocimum Basilicum extract. From t_i and t_{max} of these curves, E_i and E_{max} activation energies were

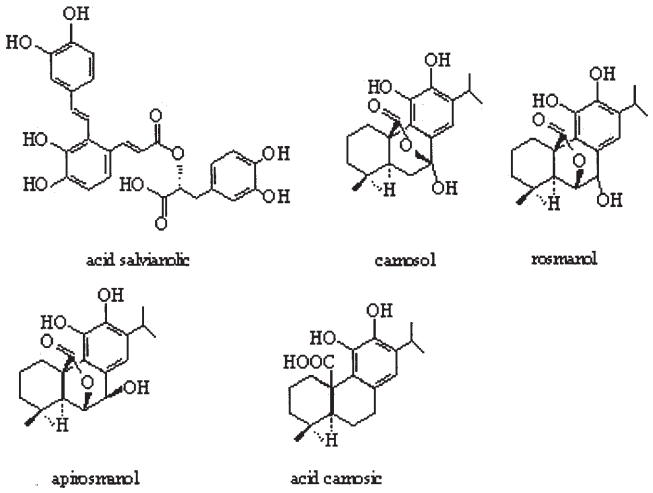


Fig. 3. Examples of flavonoids contained in the studied extracts from Lamiaceae Family

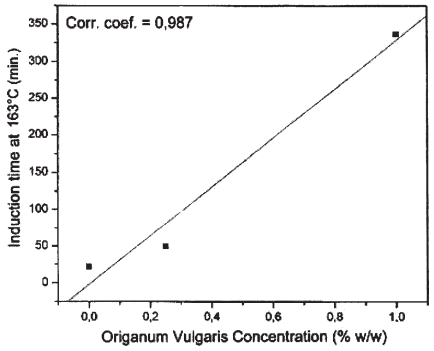


Fig. 4. The dependence of oxidation induction period with concentration extract (Origanum Vulgaris)

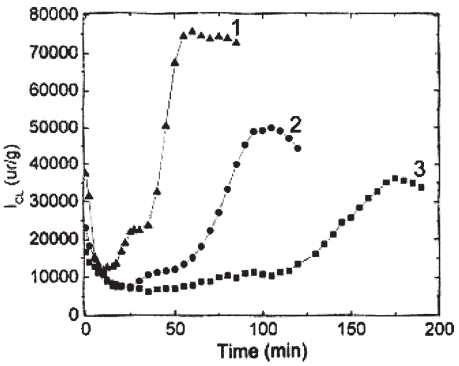


Fig. 5. CL curves for thermal oxidation of paraffin additivated (0,25% w/w) with Ocimum Basilicum.
1 – 178°C; 2 – 163°C; 3 – 156°C

calculated respectively. E_i corresponds to the induction period and E_{max} corresponds to the overall process (induction and acceleration of oxidation).

The linear dependence of lnt_{max} vs. 1/T for paraffin additivated with the same extract is presented in figure 6.

The calculated value of activation energy (E_{max}) for the oxidation process is higher in the case of the stabilized paraffin (75.3 KJ/mol) than the same energy obtained for the unstabilized ones (64.4 KJ/mol). Similar results were obtained in the case of all the other studied extracts. These higher values of the activation energy are also emphasizing the antioxidant activity presented by the natural extracts.

In the IR spectra of the natural extracts could be identified some commune bands. The vibration frequencies attributed to these bands are presented in table 3 [9].

The absorption bands from the UV-VIS spectra (table 4) are situated in the range of 210 - 310 nm and correspond to the

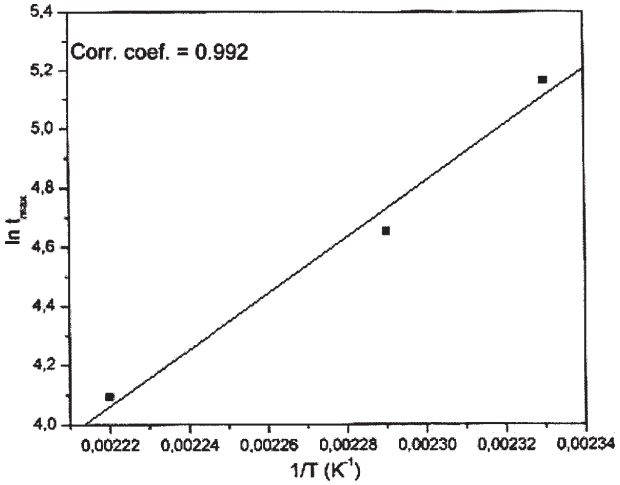


Fig. 6. lnt_{max} vs 1/T dependence for thermal oxidation of paraffin additivated (0,25% w/w) with Ocimum Basilicum

Natural extract	λ (nm)
Rosmarinus Officinalis	235,5; 284; 332
Origanum Vulgaris	237; 262; 273; 332
Ocimum Basilicum	221; 280; 329,5; 410,5; 535; 665

Table 3
THE ABSORPTION BANDS FROM THE IR SPECTRA OF NATURAL EXTRACTS (SALVIA OFFICINALIS, ROSMARINUS OFFICINALIS, RHUS TYPHIA, ORIGANUM VULGARIS AND OCIMUM BASILICUM)

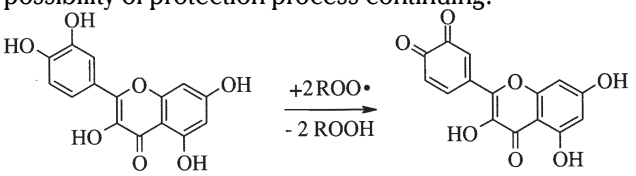
Group	ν (cm^{-1})	δ (cm^{-1})
C=C	1600 – 1625	-
OH	3200 – 3500	1010 – 1100
C-O-C (flavonoids)	-	1010 – 1100
CH_2, CH_3 aliphatic	-	1350 – 1450
CH aromatic	-	1600 – 1625

Table 4
THE ABSORPTION BANDS FROM THE UV-VIS SPECTRA OF ROSMARINUS OFFICINALIS, ORIGANUM VULGARIS AND OCIMUM BASILICUM

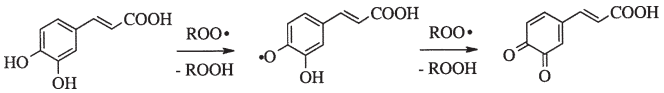
substituted aromatic rings. The values are specific to the flavonoids structure. Other bands are situated on 350 - 690 nm and correspond to the aromatic rings substituted with carbonyl groups and present an extended conjugation.

The mechanism of thermal stabilization assisted by natural extract in paraffin, which represent a standard compounds for hydrocarbon polymers in accordance with the reaction suggested in the literature data [3, 10, 11-13]. The quinonic structures result through the removal of the most active protons. The presence of other rings, other phenol substitutions and intermediate double bond allows the conjugation of neighboring bonds and a higher stability of radical intermediates is reached.

The antioxidant activity of the vegetal polyphenolic compounds from the studied extracts depends on the number of hydroxyl groups of the molecule and their substitution position on the aromatic ring. Another thing which contributes to this activity is the presence of the double bond from the C heterocycle of the flavonoid structure and the presence of hydroxyl group in the third position of catechins. In the presence of peroxy radicals the main reaction leads to the intermediate compounds stabilized to a quinonic structure which assure the possibility of protection process continuing:



In the polyphenolic acids a similar reaction is taking place and leading to stable quinonic structures:



Conclusions

Using isothermal chemiluminescence technique, IR and UV-VIS spectroscopy five different alcoholic plant extracts were studied.

The obtained results emphasise the main role of vegetal polyphenolic structures for improving the antioxidative characteristics in organic substrates. Flavonoids contained in plant extracts of Salvia Officinalis, Rosmarinus Officinalis, Rhus Typhia, Origanum Vulgaris and Ocimum Basilicum offers a real perspective to be used in food package industry.

Natural antioxidants are often used such as stabilizers of the alimentary fats and oils, cosmetics and some medicine products. Also, according to the UE recommendations, the using of these compounds can be expanded to those polymers used in the manufacturing of the food packages materials. This aspect is important with the view to avoid food products contamination with common package antioxidants which migrate by diffusion at room temperature.

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