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Estimation of the antioxidant activity of flavonoids by isothermal chemiluminescence method

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The correlation between the structural characteristics of some representative classes of flavonoids and their antioxidant activity was studied. A simple method based on isothermal CL measurements for estimating the antioxidant activity of flavonoids was used. The obtained results suggest that antioxidant activity depends both on substitution pattern of hydroxyl groups of the flavonoid skeleton and the presence of unsaturation at the C2 – C3 bond. Quercetin showed the highest antioxidant activity among tested compounds.The ability of some flavonoids to block organic peroxyl radicals was found to be comparable with butylhydroxytoluene (BHT).

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An antioxidant is any substance which is capable of delaying, retarding or preventing the progress of degradation due to oxidation, in low concentration compared with that of the oxidizing substrate. Primary antioxidants can inhibit or retard oxidation by scavenging free radicals through the donation of hydrogen atoms or electrons, which converts them to more stable products. Secondary antioxidants acts by many mechanisms including scavenging oxygen, binding of metal ions, converting hydroperoxides to non-radical species, absorbing UV radiation or deactivating singlet oxygen [1].

Flavonoids belong to the group of natural compounds that contain 15 carbon atoms in their basic nucleus that are arranged in a C_6-C_3-C_6 configuration. It means that the structure consists of two aromatic rings linked by a three-carbon unit which may or may not be a part of a third ring; conventionally, these rings are labeled as A, B and C [2, 3]. More than 5000 flavonoid components have been discovered so far and their antioxidant properties are very different.

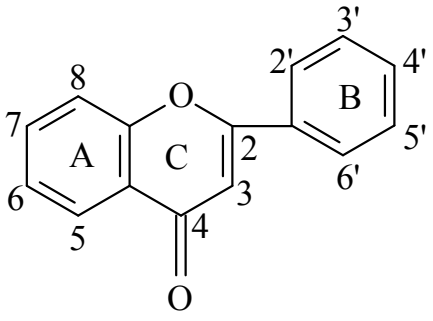


Fig. 1. Structure of flavones.

These natural products were known for their beneficial effects on health long before flavonoids were isolated as effective compounds.

Very often it is desirable to select the most effective antioxidants from a large amount of flavonoids. Several evaluation methods of the antioxidant activity for flavonoids have been developed, such as active oxygen species (superoxide anion, peroxyl radical and hydroxyl radical) scavenging capability determination [4 - 7], radical scavenging activity determination, including 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical [8,9] and 2.2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonate radical cation [10, 11] etc.

The main objective of this work is tthe comparing antioxidant behavior of some classes of flavonoids by means of isothermal chemiluminescence (CL) method. To accomplish this objective, flavonols (quercetin and morin), flavanols (epicatechin and epigallocatechin) and flavanones (naringenin and hesperitin) were subjected to CL investigations.

Experimental

The studied flavonoids (table 1) were commercially purchased. All investigated chemicals were used as received for the additivation (0.25 % w/w) of neat paraffin.

Isothermal chemiluminescence determinations were performed in air at 153°C in an oxyluminograph OL-94 instrument. Details on this equipment and on measurement procedure have been previously presented [12]. This device allows the determination of the dependence of photon counts on oxidation time. From the experimental data (fig. 2) induction onset was calculated and it was

taken as a reference parameter. This characteristic can be defined as the time before an accelerated oxidation change occurs, i.e., a measure of time before manifestation of oxidation.

Table 1. Structures of tested flavonoids.

Flavonoid	Chemical denomination	Symbol
Naringenin	5,7,4'-trihydroxyflavanone	F_1
Hesperitin	5,7,3'-trihydroxy,4'methoxy-flavanone	F_2
Epicatechin	3,5,7,4',5'-pentahydroxyflavan	F_3
Epigallocatechin	3,5,7,3',4',5'-hexahydroxyflavan	F_4
Quercetin	3,5,7,3',4'-pentahydroxyflavone	F_5
Morin	3,5,7,2',4'-pentahydroxyflavone	F_6

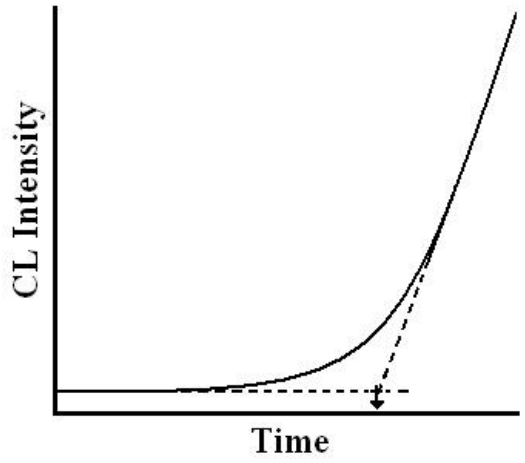


Fig. 2. Graphical method for the determination on induction oxidation time (t_i).

Results and discussion

The dependences of specific CL intensity on time for the studied flavonoids are presented in figure 3. The induction oxidation time (t_i), graphically deduced from oxyluminograms (fig. 2), and the antioxidant activity (A) are presented in table 2.

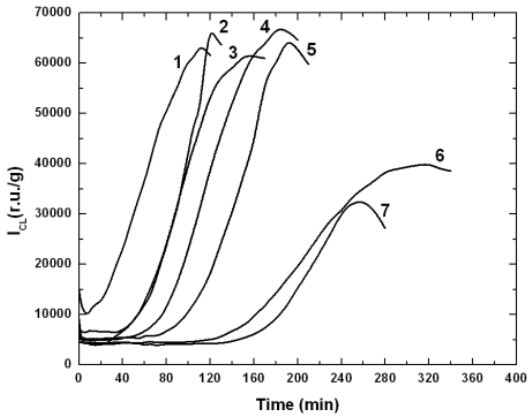


Fig. 3. Isothermal CL curves (153°C in air) from paraffin stabilized (0.25 % w/w) with the studied flavonoids. (1) blank; (2) naringenin; (3) hesperitin; (4) epicatechin; (5) epigallocatechin; (6) morin; (7) quercetin

As shown in figure 3, the presence of the flavonoid in paraffin causes a considerable increase in the induction time due to the blocking of the peroxy radicals involved in the CL emission. The flavonoid depletion in the sample is accompanied by a sharp increase in the emission as a result of the acceleration in generating chemiluminescence reactions.

Polyphenols have a well-recognized antioxidant activity. This antioxidant property has been attributed mainly to their capacity to scavenge free peroxy-radicals.

Table 2. Influence of flavonoid structure on antioxidant activity.

Family	Flavonoid denomination	Substitutions								Induction oxidation time t_i (min)	A*
		3	5	6	7	2'	3'	4'	5'		
Flavanones	Naringenin	H	OH	H	OH	H	H	OH	H	46	0.31
	Hesperitin	H	OH	H	OH	H	OH	OMe	H	52	0.36
Flavanols	Epicatechin	OH	OH	H	OH	H	OH	OH	H	61	0.42
	Epigallocatechin	OH	OH	H	OH	H	OH	OH	OH	122	0.89
Flavonols	Quercetin	OH	OH	H	OH	H	OH	OH	H	158	1.16
	Morin	OH	OH	H	OH	H	H	OH	H	129	0.94
Butylated hydroxytoluene	BHT	-	-	-	-	-	-	-	-	137	1.00
Blank	-	-	-	-	-	-	-	-	-	5	-

*Antioxidant activity (A) is expressed by the relation: $A = \frac{t_x - t_0}{t_s - t_0}$

where t_0, t_x and t_s are respectively the induction oxidation times for pure paraffin, paraffin with particular flavonoid and paraffin with butylated hydroxytoluene (BHT), which was taken as a standard.

The order of flavonoids efficiency evaluated by t_i parameter is as follows:

$$F_5 > F_6 > F_4 > F_3 > F_2 > F_1$$

Table 3 presents some evidence from reported papers on the antioxidant activity of the flavonoids studied in the present work

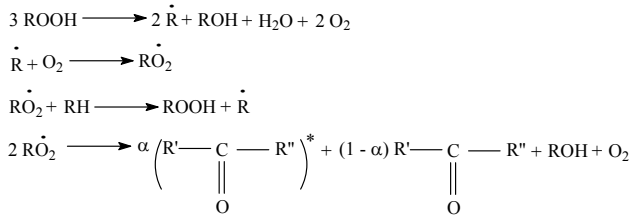
Table 3. Some cited literature data on the antioxidant activity of the flavonoids for different evaluation method

Ranked flavonoids	Evaluation method	Reference
$F_5 > F_4 > F_6 > F_3 > F_1$	The concentration of flavonoids for 50 % inhibition of lipid peroxidation	[15]
$F_6 > F_5 > F_2 > F_1$	Radical scavenging activity	[16]
$F_5 > F_6 > F_1$	DPPH radical scavenging	[17]
$F_5 > F_6 > F_1$	Inhibition of lipid peroxidation (TBA method)	[17]
$F_6 > F_4 > F_3 = F_1 > F_2$	Amount of Triton X-100 required to attain 50 % of the maximum fluorescence intensity	[18]
$F_5 \geq F_6$	Inhibition of heat-induced oxidation in β-carotene-linoleic acid system	[19]
$F_5 > F_1$	μmol DPPH scavenged per μmol flavonoid	[20]
$F_5 > F_6$	Trolox equivalent antioxidant capacity (TEAC)	[11]
$F_4 > F_3$	Inhibition of linoleic acid per oxidation	[21]

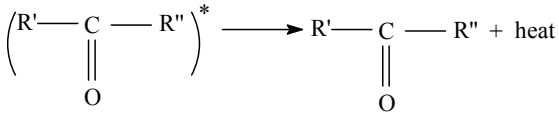
The data listed in table 3 confirm the validity of the flavonoid efficiency order obtained by the isothermal chemiluminescence method.

The chemiluminescence analysis indicates the following considerations:

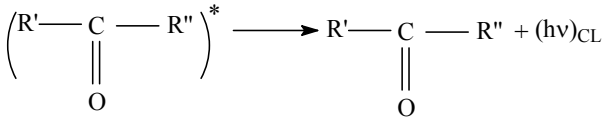
(a) The degradative oxidation of organic substrates involves peroxides and free radicals:



The above equations show a free-radical chain process that terminates with the production of an electronically excited ketone, a ground state ketone, an alcohol, and oxygen. The excited ketone goes to the stable ground state giving off heat (and no light):



or light (and no heat):



Normally most of the energy is released as heat and only a small fraction of the excited molecules emit CL.

(b) Naringenin and hesperitin hydrogenated between C_2 and C_3 (so-called flavanone) have showed a weak antioxidant activity. This result indicated that the presence of double bond at C_2 and C_3 was essential for the antioxidant activity;

(c) The level of antioxidant activity increased in correlation with the number of OH groups when hydroxyl derivatives are with 2 to 5 OH groups. The compound with 6 OH groups has indicated the tendency to a little decline;

(d) On the basis of CL results it appears that the most effective radical scavengers are flavonoids with 2',4'- or 3',4',-dihydroxy substitution pattern on the B-ring and hydroxyl group at the C_3 position;

(e) Quercetin has showed the highest antioxidant activity among the tested compounds. The part of the antioxidant effect of quercetin is due to the structural features of C-ring. Quercetin has a double bond between carbons 2 and 3 in the C-ring as well as a ketogroup in C_4. These structural features improve the antioxidant effect of quercetin due to an effective hydrogen donation;

(f) The presence of a *o*-dihydroxy structure on the B-ring confers a higher degree of stability on the flavonoid phenoxyl radicals (fig. 4)

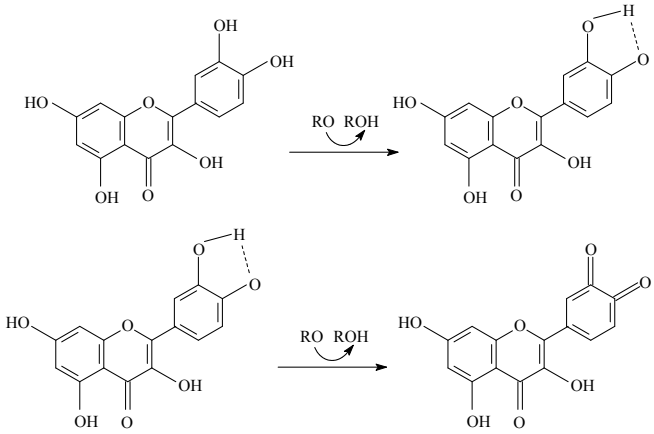


Fig. 4. Antioxidant action mechanism of 3',4'-diOH flavonoids

It is interesting to note that a consecutive two-electron oxidation was postulated [22] as the mechanism of the

peroxyl radical reaction with 3',4'- and 2',5'-dihydroxyflavonols to yield corresponding quinines. The first one-electron oxidation produces the flavonoid phenoxyl radical, which subsequently scavenges another peroxyl radical. Apparently, organic peroxyl radicals selectively attack the B-ring of any 3',4'- or 2',5'-dihydroxyflavonoid.

On the other hand, the 2,3 double bond in the C-ring in conjungation with the carbonyl in the C_4 improves electron delocalization, which stabilizes the antioxidant radical.

(f) The ability of some studied flavonoids to block peroxyl radicals was found to be at least comparable, if not better than that of conventional phenolic antioxidants such as butylhydroxytoluene (see table 2). Synthetic antioxidants have restricted use in food packaging as they are suspected to be carcinogenic. Therefore, the importance of search natural antioxidants has greatly increased in the recent years [23]

Conclusions

Flavonoids can be quantitatively examined for their antioxidant effectiveness against oxidation using isothermal CL method.

The lengths of the CL induction correlated with the number of hydroxyl groups in the molecule. At the same number of hydroxyl groups, epicatechin had a small effect while quercetin had the highest protective effect of all the flavonoids tested.

A substance with antioxidant activity is itself a target for oxidation; 3-hydroxy flavonols have the preferred site of attack in the structure of B-ring with the initially formed semiquinone stabilized by 2,3-double bond.

The antioxidant activity measurements by isothermal chemiluminescence are confirmed by other suitable methodologies. The CL method can be used for screening flavonoid antioxidants and estimating the antioxidant activity.

References

[1] M. H. Gordon, Measuring antioxidant activity. In: J. Pokorny, N. Yanishlieva, M.H. Gordon (Eds), Antioxidants in food: practical applications, pp. 71-84, Cambridge: Woodheart Publishing Limited, 2001.

[2] J. B. Harborne, The flavonoids: recent advances. In: Goodwin T. W., (Ed), Plant Pigments. London, England, Academic Press, 1988, P. 299-343.

[3] J. J. Macheix, A. Fleuriet, J. BILLOT, Fruit Phenolics Boca Raton, USA, CRC Press, 1990.

[4] S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M. G. Simic, J. Am. Chem. Soc. **116**, 4846 (1994).

[5] Y. Hanasaki, S. Ogawa, S. Fukui, Free Radic. Biol. Med., B, p. 845 (1994).

[6] A. Arora, G. Nair, G. M. Strasburg, Free Radic. Biol. Med **24**, 1355 (1998).

[7] W. Bors, C. Michel, Free Radic. Biol. Med. **27**, 1413 (1999).

[8] M. S. Blois, Nature **181**, 1199 (1958).

[9] F. Nanjo, K. Goto, R. Seto, M. Suzuki, M. Sakai, Y. Hara, Free Radic. Biol. Med **21**, 895 (1996).

[10] C. A. Rice-Evans, N. J. Miller, G. Paganca, Free Radic. Biol. Med. **20**, 933 (1996).

[11] D. Ivecovic, S. Milardovic, M. Roboz, B. S. Grabaric, Analyst **130**, 708 (2005).

[12] S. Jipa, T. Zaharescu, R. Setnescu, T. Setnescu, M. J., S. Brites, A. M. G. Silva, A. J. Marcelo-Curto, B. Gigante, Polym. Int. **48**, 414 (1999).

[13] W. Bors, W. Heller, C. Michel, M. Saran, Methods Enzymol. **186**, 343 (1990).

[14] K. E. Heim, A. R. Tagliaferro, D. J. Bobilya, J. Nutr. Biochem. **13**, 572 (2002).

[15] B. Yang, A. Kotani, K. Aral, F. Kusu, Analytical Science **17**, p. 599 (2001).

[16] D. Amic, D. Davidovic-Amic, D. Beslo, N. Trinajstic, Croatica Chemica Acta **1**, 55 (2003).

[17] M. Furusawa, T. Tanaka, T. Ito, A. Nishikawa, N. Yamazaki, K. Nakaya, N. Matsuura, H. Tsuchiya, M. Nagayama, M. Iinuma, Journal of Health Science **51**, 376 (2005)

[18] P. J. Oteiza, A. G. Erlejman, S. V. Verstraeten, C. L. Keen, C. G. Fraga, Clinical and Developmental Immunology, **12**, p. 19 (2005)

[19] O. Farcas, J. Jakus, K. Herberger, Molecules **9**, 1079 (2004)

[20] M. M. Silva, M. R. Santos, G. Caroco, R. Rocha, G. Justino, L. Mira, Free Radic. Res. **30**, 1219 (2002)

[21] Z. S. Jia, B. Zhou, L. Yang, L. M. Wu, Z. L. Liu, J. Chem. Soc. Perkin Trans **2**, 911 (1998)

[22] N. Uri, In: Autooxidation and Antioxidants; Lundberg, W.O. (Ed), Interscience, London, 133-169 (1961).

[23] G. K. Jayaprakasha, T. Selvi, K. K. Sakariah, Food Res. Int. **36**, 117 (2003)

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