Application Note 01

## EPR-spectroscopy in free radicals and antioxidants chemistry





Developing new and optimizing existing methods of antioxidant activity determination is an important challenge facing the pharmacy, cosmetics and food industries. Interest in antioxidant activity measurements of various products proves the importance of organism protection against free radicals [1].

Antioxidants, both natural and synthetic, are used as food additives to prevent products oxidation [2], i.e. to inhibit their lipid peroxide oxidation. Among them are the polyphenols and their byproducts (phenolic acids, flavonoids, proantocyanidines, prenylflavonoid, tannins and aminophenols) are the most important antioxidants [3].

Several factors influence the antioxidants efficiency such as: chemical structure, concentration, temperature, oxidation substrate type and system physical state, presence of prooxidants and synergists [4].

Antiradical activity (ARA) research methods differ by oxidation source, the oxidation substrate and way of oxidation process measurement. Various ARA measurement methods have developed, been however, EPR-spectroscopy is the only analytical approach capable of specific free radicals determination. The EPR technique based is on measurements of free radicals EPR-spectrum intensity during their interaction with antioxidants [5].

EPR technique is widely used for antioxidants evaluation in olive oil [6], tea [7], coffee [8], beer [3], wine [9], other alcoholic beverages [5], fruit juices, soft drinks [10] and honey[11]. Food products ARA can be evaluated using the following stable radicals:

- galvinoxyl radical (Galv-O •);
- DPPH• The rate of its signal intensity loss is determinative for the antioxidant ability to bind the DPPH radical;
- ABTS+• cation-radical;
- nitroxyl radical (TEMPOL hydroxy-2,2,6,6tetramethylpiperidine N-oxyl).

One of the most perspective and widely applied approaches to ARA studies of natural and synthetic antioxidants is the reference-free EPR-spectroscopy method based on DPPH

The rate of DPPH neutralization or the intensity loss in the final point may be indicative for the antioxidant ARA.

radical [12].



То demonstrate the EPR application for studying the antioxidant potential of food oils several samples of commercial olive oil have been analyzed. The antioxidant potential of food oil is the result of natural antioxidants present it, phenolic compounds and tocopherols being the most important ones. The DPPH inhibition by antioxidants in olive oil was observed by the rate of signal intensity loss (fig.1). In order to estimate the ARA 100ul olive oil was added to 1ml DPPH solution. As seen from the plot, 30 minutes is enough for most of the reaction to proceed (fig.2).



## Figure 1 - DPPH EPR signal inhibition by olive oil antioxidants

Experiment parameters: center field 337.6mT; sweep width 9mT; modulation frequency 109.375kHz; modulation amplitude 400uT; attenuation 12dB; number of points 500; sweep time 60s; number of scans 1.



Numerous examples of EPR-based experimental research of free radicals and antioxidants demonstrate that EPR is a simple, robust and reliable method of ARA estimations in food products. The EPR spectroscopy can be used for deep scientific explorations of various factors influencing the ARA which is of great practical importance for food producers as well as for consumers.



спектроскопии. Магистерская диссертация. // Уральский федеральный университет им. Б.Н. Ельцина. –Екатеринбург. –

F. Shahidi, Y. Zhong. Vol. 141 (192). P. 3042-3049
F. Shahidi, Y. Zhong. Measurement of antioxidant activity. // Journal of functional foods. –2015 –Vol. 18 –P. 757–781
M. Bartoszek, J. Polak. An electron paramagnetic resonance study of antioxidant properties of alcoholic beverages. // Food Chemistry. –2012-Vol. 132. – P.2089–2093
P. Dais, D. Boskou. Detection and Quantification of Phenolic Compounds in Olive and Biological Eurids. // Olive Oli-

Compounds in Olive Oil, Olives, and Biological Fluids. // Olive Oil: Minor Constituents and Health. – P. 55-106

7. M. Polovka, V. Brezova, A. Stas<sup>\*</sup>ko. Antioxidant properties of tea investigated by EPR spectroscopy. // Biophysical Chemistry – 2003. Vol.106 - P. 39-56

antioxidants: An EPR study. // Food Chemistry – 2009. – Vol. 114. – P. 859-868

9. G.J. Troup, D.R. Hutton, D.G. Hewitt, C.R. Hunter. FREE RADICALS IN RED WINE, BUT NOT IN WHITE? // Free Rod. Res.. –

of fruit juices, drinks and nectars, as determined by EPR and UV-vis spectroscopies. // Spectrochim. Acta Part A Mol. Biomol. Spectrosc. –2016. –Vol.153 –P. 546–549

M. Zalibera, A. Stasko, A. S. Lebodova, V. Jancovicova, T. Cermakova, V. Brezova. Antioxidant and radical-scavenging activities of Slovak honeys – An electron paramagnetic resonance study. // Food Chemistry.–2008.–Vol. 110–P. 512–521

12. L. LUNGUI, C. POPA, M. MARINESCU, V. TECUCEANUI, A. FLORIN DANET, V. BERCU ANTIOXIDANT CAPACITY OF SOME CALENDULA EXTRACTS BY EPR SPECTROSCOPY // Romanian Reports in Physics. – 2019–Vol. 71, 706