

EPR investigation of antioxidant characteristics of some irradiated natural extracts

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The aim of the present work is to study the antioxidant characteristics of some irradiated natural extracts, like white Seaberry (*Hippophae Rhamnoides*) and Spirulina (*Spirulina Platensis*) using EPR (Electron Paramagnetic Resonance) spectroscopy technique. The methods used in this research concern the utilization of stable free radicals from nitron classes, like nitroxide radical Tempol (*2,2,6,6-tetramethyl-4-hydroxypiperidine-oxyl*) and spin trap PBN (*N-t-Butyl- α -phenylnitron*). The purpose is to see which are the changes induced in antioxidant activity by irradiation process, depending on irradiation absorbed doses. For low adsorbed doses was observed an increasing of antioxidant activity for all studied samples.

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1. Introduction

Oxidative processes occurring in living organisms result in the formation of highly reactive free radicals that are known to cause accelerated aging of living cells and to increase the risk of health impairment, including initiation of cancer [1]. Antioxidants are molecules that can safely interact with free radicals and terminate chain reaction before vital molecules are damaged. Consequently, naturally occurring antioxidants are receiving increased attention in biological research, medicine, and pharmacy and food technology [2].

The most studied antioxidants are natural extracts from plants, focused especially on vegetables and medicinal herbs. White Seaberry has been shown to have a potent antioxidant activity mainly attributed to its bioflavonoid and vitamin C content [3,4]. Spirulina, the green microscopic algae, provide high concentrations of amino acids and the extracts of Spirulina present radioprotective effects [5,6]. Also, polyphenols and flavonoids from green tea [7,8] and grapes seeds and peels [9] have demonstrated significant antioxidant characteristics in numerous studies.

The aim of the present work is to study the antioxidant characteristics of irradiated natural extracts, like white Seaberry and Spirulina, using EPR spectroscopy technique. The methods used in this research concern the utilization of stable free radicals from nitron classes, like nitroxide radical Tempol and spin trap PBN.

Nitroxide radicals and spin traps are utilized in measuring free radicals activity because they react with free radicals both in vitro and in vivo, producing stable complexes that can be measured by a variety of techniques [10-12].

The purpose of this work is to see which are the changes induced in antioxidant activity by irradiation process, depending on irradiation absorbed doses. The

concentrated natural extracts in powder forms were irradiated with different absorbed doses. For low adsorbed doses was observed an increasing of antioxidant activity for all studied samples.

2. Experimental

White Seaberry (*Hippophae rhamnoides*) and blue-green algae *Spirulina Platensis* were purchased from Hofigal S.A., which is the main Romanian manufacturer of natural products. Powder samples of natural extracts were irradiated to different absorbed doses (1kGy \div 25kGy) in plastic package at room temperature and atmospheric pressure, in accelerated electron beams from a linear electron accelerator, with a medium energy of 6 MeV. It was used the electron accelerator from "National Institute of Laser, Plasma and Radiation Physics, Electron Accelerator Laboratory" from Bucharest-Magurele, Romania. After radiation treatment these samples were exposed to a physical process of extraction, in order to obtain liquid samples. The used solvent was Methanol.

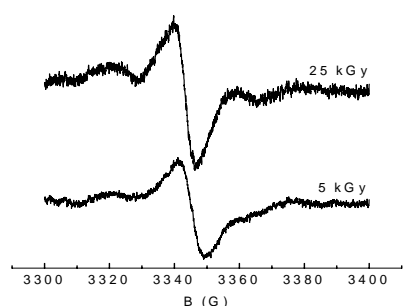
A solution of ($9 \cdot 10^{-5}$ Molar) Tempol was added in liquid samples of white Seaberry and Spirulina. Samples were measured using 20 cm length, 1 mm inside diameter quartz capillary. EPR spectra were recorded at room temperature with a portable EPR Spectrometer "ADANI portable EPR Spectrometer PS8400", Resonance Instruments Inc., operating in X-band (9.1 GHz \div 9.6 GHz) equipped with a computer acquisition system. The spectrometer settings used for the experiments were as follows: modulation frequency 100 kHz; sweep width 100 G; sweep time 20s; receiver gain 5×10^1 for white Seaberry, and 1×10^2 for Spirulina; number of data points, 4096. Using the same parameters, the spectra were recorded at different time intervals (40 to 40 seconds).

The variations of the relative concentration of radical species were obtained through double integration of

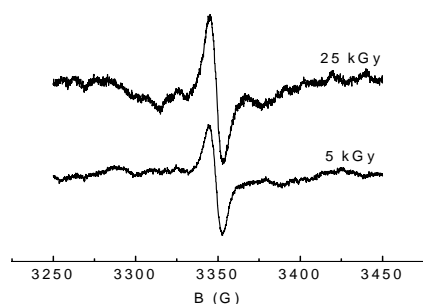
experimental spectra. Time dependence curves of the concentration of radical species were used in order to characterize the antioxidant activity of studied samples.

3. Results and discussion

The EPR spectrum of electron beam irradiated white Seaberry and Spirulina (Fig. 1) in solid state represent a sum of individual spectra corresponding to all free radicals simultaneously present in the samples or the same free radicals localized in various local environments. In both cases, the spectrum sample is dominated by a broad central signal with specific characteristics given by chemical structures. By increasing the irradiation dose the intensity of the EPR signal increased too, shown that the number of radicals formed depending on the absorbed dose.



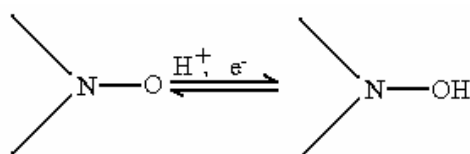
a



b

Fig. 1. EPR spectra of irradiated white Seaberry (a) and Spirulina Platensis (b) in solid state.

We have investigated the antioxidant activity of white Seaberry using a commonly encountered nitron spin label, nitroxidic radical Tempol (2,2,6,6,-tetramethyl-4-hydroxypiperidine-oxyl). The nitroxide radicals are relatively stable towards oxidation but they can be easily reduced to the corresponding hydroxylamines (Scheme 1).



Scheme 1.

The standard redox-potential of piperidine nitroxide derivatives ($E_0 = 0.2$ eV) is high enough to oxidise biological compounds such as polyphenolics, ascorbic acid, semiquinones and superoxide radicals [13].

In case of white Seaberry/Tempol complex in liquid state the number of paramagnetic species presents a decrease in time with different rates, depending on the absorbed doses. From decay of EPR signal caused by the reaction between polyphenolics compounds and Tempol it is possible to draw conclusions about antioxidant activity of this sample.

The antioxidant activity in experimental white Seaberry samples irradiated with different absorbed doses was found to depend on absorbed doses. For low adsorbed doses was observed an increasing of antioxidant activity for all studied samples. At high-absorbed doses the antioxidant activity decreases.

The decay rate of reaction between polyphenolics compounds and Tempol has monitored by using double integrated residual EPR signal (Fig. 2). The experimental time dependencies of TEMPOL relative concentration represent exponential functions according to the formal first order kinetic model. The best fit was obtained using the following first order exponential decay:

$$I(t) = I_0 + A \cdot e^{-\alpha \cdot t}$$

in which I_0 and A are parameters depending on experimental EPR conditions. The parameter α , is constant rate of nitroxide radical decay, depending on the quality of polifenolic content of these samples. This parameter can be taken as characteristic factor to evaluate the oxidant capability of all samples irradiated with different absorbed doses (Fig. 3).

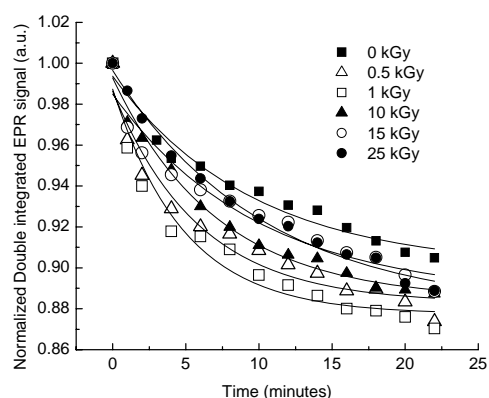


Fig. 2. Decay rate of EPR signal of white Seaberry/Tempol complex in liquid state.

From (Fig. 3) it can be concluded that the antioxidant activity of white Seaberry increase at low absorbed doses and decrease for high absorbed doses. Antioxidant activity of white Seaberry is maxim between around 1 kGy. For high-absorbed doses (above 5 kGy) an important decrease in time of antioxidant activity is induced. The relative

errors for all measurements were about 10%. Similar results were obtained in case of *Spirulina Platensis*.

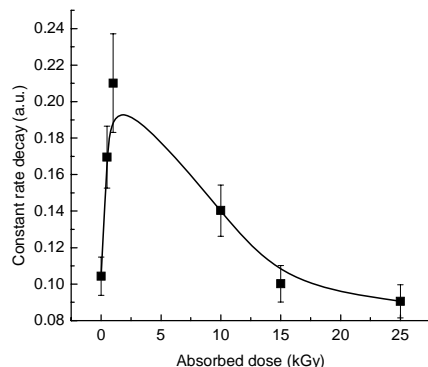
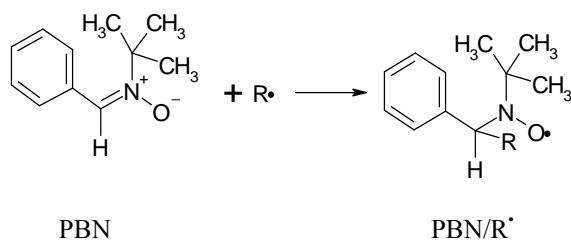


Fig. 3. Antioxidant activity of white Seaberry vs. absorbed doses.

The antioxidant activity of *Spirulina (Spirulina Platensis)* was investigated using N-t-Butyl- α -phenylnitron (PBN) as spin trap. PBN is a stable compound and forms relatively long-lived spin adducts with various types of radicals as in (Scheme 2).



Scheme 2. Formation of spin adduct with PBN

In order to immobilize in one matrix the free radicals present in these samples the *Spirulina* /PBN complex was applicate on silicagel. Experiments in the presence of PBN spin trap gave complex results (Fig. 4).

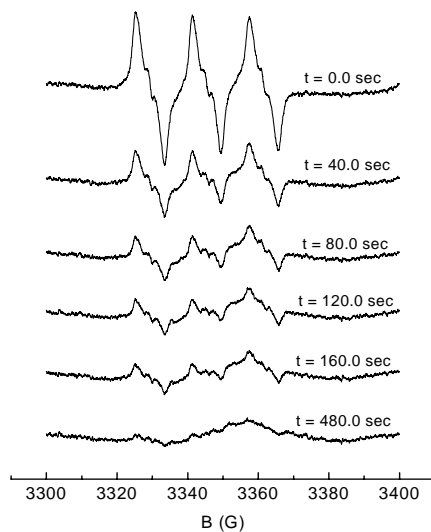


Fig. 4. The variation of PBN EPR signal in non-irradiated *Spirulina Platensis* on silicagel.

These spectra represent a sum of individual spectra corresponding to different species of free radicals simultaneously present in the studied samples. From EPR spectra analysis can be assumed that the dominant reactants presents in this system are hydroxyl radicals (OH^\bullet). The presence of these species was not properly observed by classical methods, but their presence is motivated by the fact that *Spirulina* have hydroscopic characteristics. The formed adduct had hyperfine splitting constants of $a_N = 16.2$ G and $a_H = 3.1$ G.

The EPR spectra of *Spirulina*/PBN complex were recorded at different time intervals using the same parameters. The intensity of EPR signal was found to decrease in time, showing that *Spirulina Platensis* present powerful antioxidant characteristics. The variations of the relative concentration of radical species were obtained through double integration of experimental spectra.

Decay rate of EPR signal of non-irradiated *Spirulina*/PBN complex on silicagel is presented in (Fig. 5). Absorbed doses dependence of antioxidant activity of *Spirulina* is similar with the one obtained in case of white Seaberry [5].

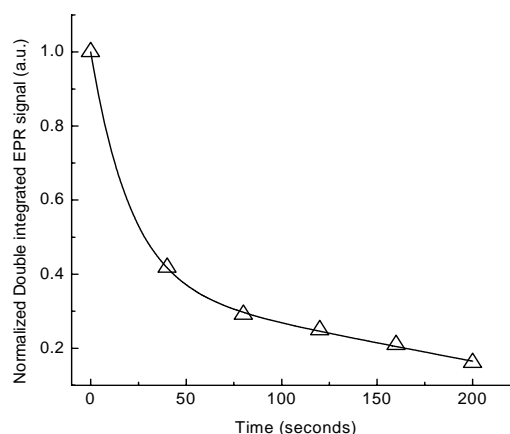


Fig. 5. Decay rate of EPR signal of non-irradiated *Spirulina*/PBN complex on silicagel.

4. Conclusions

The proposed methods for qualitative analysis of antioxidant activity of some natural extracts are based on monitoring reduction of spin trap, respectively nitroxide radical molecules using EPR spectroscopy. From curve fitting of decay rate of stable free radicals from nitronne classes, like nitroxide radical Tempol and spin trap PBN, it is possibly to evaluate the main antioxidative characteristics of natural extracts.

The purpose of this work it was to see which are the changes induced in antioxidant activity by irradiation process, depending on irradiation absorbed doses. From the obtained experimental data it can be assumed that for low adsorbed doses the antioxidant activity increase for all studied samples. Antioxidant activity of white Seaberry is maxim around 1 kGy. For high-absorbed doses (above 5

kGy) an important decrease in time of antioxidant activity is induced. This behaviour is probably due to possible structural modifications of chemical compounds from studied natural extracts that have antioxidant characteristics.

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