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IDENTIFICATION OF GAMMA IRRADIATED LYOPHILIZED FOREST FRUITS BY EPR SPECTROSCOPY

I. NACHEVA[1], K. ALEKSIEVA[2], K. DIMOV[1], D. MITEVA[1] and Tsv. TSVETKOV[1]
[1] *Institute of Cryobiology and Food Technologies, BG - 1407 Sofia, Bulgaria*
[2] *Bulgarian Academy of Sciences, Institute of Catalysis, Molecular Catalysis with Center of EPR Spectroscopy, BG - 1113 Sofia, Bulgaria*

Abstract

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The objective of the present study was to prove the radiation treatment of a set of lyophilized forest fruits (blackberry, blueberry, aronia, strawberry and black elder), irradiated with doses of 2 and 4 kGy by the method of Electronic Paramagnetic Resonance (EPR). A low intensive singlet line was registered for all samples before the irradiation. The results show that after irradiation the intensity of the natural signal is increased, as the so-called "cellulose like" EPR spectrum was registered only for aronia. From the studied lyophilized fruits, according to the protocol for irradiated foods, containing cellulose (EN 1787), and radiation treatment can be proved only for aronia. Protocol EN 13708 for irradiated foods, containing crystalline sugar is not applicable for these samples.

Key words: EPR spectroscopy, gamma irradiation, lyophilization, forest foods

Introduction

According to a number of classifications, forest fruits are among the most useful foods and this estimable position is not by chance. Besides their exquisite taste, what unite them are the benefits they bring for the organism. They are an excellent source of vitamin C, as well as of iron, calcium, vitamins of the B group and manganese. They are rich of polyphenol compounds, which help the brain to fulfill important supporting functions. They are one of the strongest antioxidants, surpassing most of the fruits and vegetables.

The investigations show that people, whose nutritional regime is rich of flavonoids, contained in forest fruits are less in danger to develop Parkinson's disease. Flavonoids have a protective effect against disorders related to the ageing processes; they lower the risk of heart diseases, high blood pressure and some cancer types. According to the conclusions of the scientists, the anthocyanins, a subclass of the flavonoids, have a neuroprotective effect. It has been proved that forest fruits are beneficial for improving of the brain functions and for preventing of memory loss, related to age. The consumption of fruits has a favorable effect on the brain signal paths, in cases of inflammation and cell death. The effect of these improvements of the brain function can inhibit the development of age related brain diseases as Alzheimer's disease and dementia. The aronia juice is rich of biologically active substances. It contains vitamins P, E, C, K, B_2 and the microelements manganese, iron, etc. The iodine content is 3-4 times greater compared to other fruits, which makes aronia especially precious in cases of iodine deficit, for normalizing of the thyroid gland functions. The red berry - a tasty and fresh treasury of vitamin C dietetic fibers is a "cleaner" of the bad cholesterol and stabilizer of the blood pres-

E-mail: iliana_nacheva@abv.bg

sure. The black elder fruit are rich of anthocyan compounds, helping for the neutralizing of the free radicals. Besides that, they contain a lot of pectin and cellulose. They also have a laxative, diuretic action and aid lowering of high temperature, which is predominantly due to the glycoside substances. May be the greatest plus for human health from the consumption of blackberries is the considerable quantity of phenolic acids in them, which are antioxidant compounds known as strong anticarcinogenic agents and many other beneficial for the health qualities as well.

The phenols in blackberries include anthocyanins, ellagic acid, rutin (vitamin P), cinnamic acid, as well as substantial quantities of the antioxidant vitamins A and C. The folic acid in the composition of strawberries protects the fetus in the mother's womb against inborn defects; vitamin C efficiently prevents the development of common cold and infectious diseases.

Forest fruits are consumed fresh and processed. They are stored at low temperatures (0-3° C), under refrigerator conditions, by freezing, drying and by preservation. Their safe and long-term storage necessitates the applying of effective methods for preservation, which prolong the storage term with maximum preserving of their taste qualities. Such modern, high technology methods are freeze-drying and gamma sterilization. The freeze-drying method, combining two processes - freezing and drying under vacuum, allows to avoid the disadvantages of all other methods of preservation, as the end products feature preserved initial qualities - color, aroma, nutritional properties, vitamins content, unchanged initial volume, fast rehydratation (Mladenova and Yordanov, 2007). The gamma irradiation, also called cold sterilization, is a process by which is achieved an elimination or a considerable decrease of the number of the potentially dangerous for the consumers' health pathogenic microorganisms contained in the products and their storage term is considerably prolonged.

The lyophilization of foods, including fruits is a costly process but in some extreme conditions, the use of this technology is exigent. That is why the interest in lyophilized foods is growing and at the same time, the investigations by EPR spectroscopy of irradiated lyophilized foods are insufficient (Aleksieva et al., 2009; Bogl et al., 1988; Re et al., 1999; Yordanov et al., 2006).

Free radicals are part of the processes in the organism. They participate in the digestive processes, as well as in the supporting of the immune system. However, under certain conditions, when their quantity increases considerably, damage of some cells may occur (Bayram and Delincee, 2004). As the free radicals are of paramagnetic nature, they can be registered by the method of EPR spectroscopy.

EPR is a field of spectroscopy, where the electromagnetic radiation with microwave frequency is absorbed by the atoms of the molecules or solid bodies with electrons with non-paired spins. Therefore, by EPR can be studied: free radicals in solid, liquid and gas state; point defects in solid bodies; biradicals, atoms and ions with unfinished inner electronic layers - of the transition metals, the rare earth elements and the actinides. (Vertz and Bolton, 1975).

During the last two decades foods irradiation has been recognized as a fast, cost effective and secure method for improving of their quality and prolonging of their shelf life. Nowadays, the radiation treatment is legally permitted in above 40 countries all over the world for above 60 different food kinds. For commercial purpose in the European Union, most foods are irradiated in Belgium, France and the Netherlands (Kume et al., 2009). The applying of high-energy radiation imposes the use of fast and effective methods for proving of a preceding radiation treatment. Until the present moment the European Committee for Standardization (EN) has adopted 10 standards, of which six basic and four «screening" (EN 1786, 1997 EN 1787, 2000, EN 13708, 2001). Three of the basic standards for identification of irradiated foods use the method of the Electronic paramagnetic resonance. In foods are investigated the naturally content radicals as well as such generated as a result of irradiation with γ–rays (Nikolova et al., 2007; Gancheva and Yordanov, 2007; Yordanov and Aleksieva, 2009; Yordanov and Pachova, 2006). The lyophilized foods are irradiated with the purpose to prolong their storage term.

The objective of the present study was to investigate various lyophilized forest fruits (blackberry, blueberry, aronia, strawberry and black elder) before and after irradiation with doses of 2 and 4 kGy by the method of the EPR spectroscopy.

Materials and Methods

Subject of the investigation are forest fruits - aronia, blueberry, black elder and strawberry, technologically processed in ICFT. The samples were distributed in three groups, as follows:

First group – control – not irradiated
Second group – irradiated with 2 kGy
Third group – irradiated with 4 kGy.

The three groups' forest fruits were studied immediately after the irradiation as well as after their 6 months storage. The investigation was made on spectrometer JEOL JES FA-100, as well as on BRUKER, model ER 200 D SRC, working in the X-range, according to EN 1787 -2000.

The parameters of the EPR measurements were equal for all samples except for the amplification of the spectrometer and the scanning interval:

1. Centre of the magnetic field – 347 mT
2. Modulation amplitude – 0.5 mT
3. Microwave power – 1 mW

The freeze-drying was carried out under vacuum conditions in a freeze-drying installation of the company "Hochvakuum-TG –16.50" with contact plates heating. The irradiation was carried out in gamma irradiation installation "Gamma-1300" with radiation source Cs^{137}, with dose power 1.43 Gy/min.

Results and Discussion

Before the irradiation in all analyzed samples was registered a low intensive singlet line with g = 2.0048 (Figure 1a). This line is due to naturally present free radicals with semihinon structure, formed by the oxidation of fatty acids in the plants and included in the lignin (Yordanov and Aleksieva, 2009).

After irradiation, the singlet line intensity for all investigated sample increased with increasing of the irradiation dose, as the g-factor did not change (Figure 1b). All samples were also detected after a tenfold increase of the EPR spectrometer amplification. Only for aronia samples were registered typical «cellulose-like» EPR spectra, owing to generated free radicals in the cellulose because of irradiation with 2 and 4 kGy. By arrows are marked (Figure 1c) the co called «satellite lines», whose registration is a sufficient proof for radiation treatment according to the Protocol (EN1787) of the European Committee for Standardization. As it is seen on Figure 1c in the irradiated aronia samples, these lines are with very low intensity even after a tenfold increase of the spectrometer amplification (on Figure 1c it is seen that the central line is cut). For all other samples the so called «cellulose-like» EPR spectrum was not detected and therefore according to Protocol EN 1787 it cannot be asserted if the samples are irradiated or not. On the other side, «sugar-like» spectra were not detected, in whose presence according to Protocol EN 13708 it can be asserted that the samples are irradiated. The above two protocols of the European Committee for Standardization are basic and refer to irradiated foods of plant origin, studied by EPR spectroscopy. According to them in the absence of «cellulose-like» and «sugar-like» EPR spectra a radiation treatment cannot be proved. Similar results were also obtained by the same laboratory (Yordanov et al., 2006) from a previous EPR analysis of lyophilized foods. A typical sugar-like spectrum was obtained only for lyophilized apricot. Yet the g-factor of the registered complex spectra differs before and after irradiation, which then gave us grounds to prove irradiation in the studied fruits (Yordanov et al., 2006). For the present samples (except for aronia) was registered only a singlet line whose intensity increases

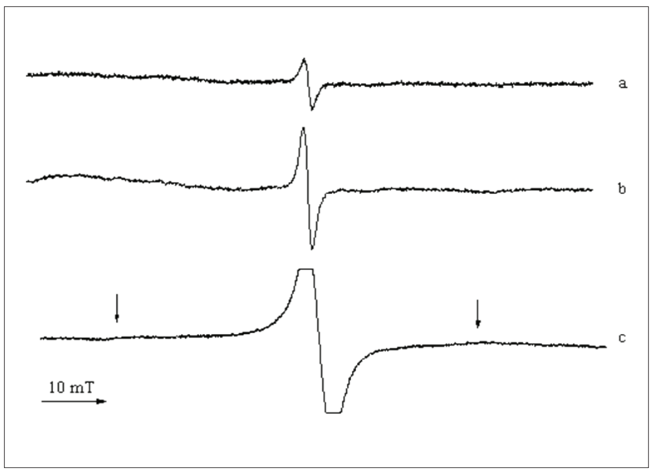

Fig. 1. EPR spectra of lyophilized forest fruits before and after irradiation

after irradiation, but a change in the g-factor was not found, i.e. it cannot be asserted that the samples are irradiated (Figure 1).

For lyophilized blueberry besides the central singlet line were registered in addition six lines (marked on Figure 2c with *), which is due to the presence of Mn^{2+} ions. The six super fine lines are a result of the naturally present Mn^{2+} ions in the plants (the nuclear spin of ^{55}Mn is 5/2). Manganese is an essential element. It is accumulated from the soil in the plants where it participates in the process of photosynthesis. In a previous EPR analysis of samples of white and black pepper, savory, dill and peppermint were also registered ions of Mn^{2+} (Yordanov and Aleksieva, 2004).

For the lyophilized blueberry, the manganese spectrum, which is radiationally insensitive, can be used as an internal standard to prove radiation treatment (Yordanov and Aleksieva, 2004). This approach is not included in the protocols of the European Committee for Standardization, but it has been developed in previous studies of our laboratory. As it can be seen on Figure 2 the intensity of the singlet signal increases with the irradiation dose, as this line is radiationally sensitive, while the intensity of the manganese lines remains constant. In such a way, the correlation of the lines Mn^{2+}/singlet can serve for proving of the radiation treatment.

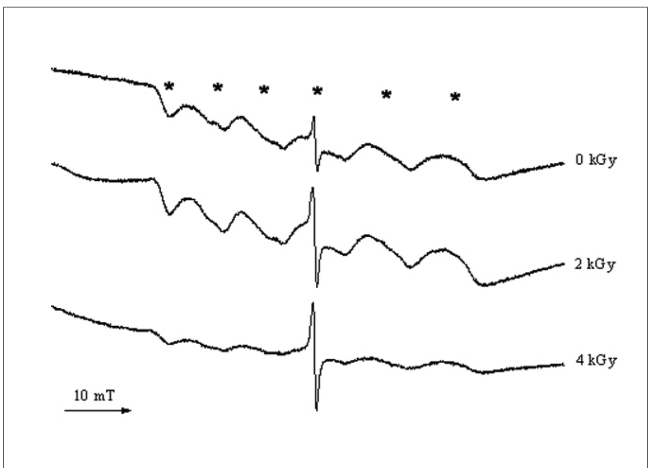

Fig. 2. EPR spectra of lyophilized blueberry before and after irradiation

The EPR spectra of fruits irradiated with 2 and 4 kGy after 6 months storage were investigated. A fall of the singlet line intensity and disappearing of the satellite lines for the aronia were observed.

Conclusions

By EPR it has been established that in the irradiated aronia samples is observed the so-called "cellulose-like" spectrum, whose registration is a sufficient proof of radiation treatment. In samples of blueberry, elder, strawberry and blackberry only and increase of the singlet line intensity with increasing of the dose is observed, which according to Protocol EN 1787 is not a sufficient condition for proving of radiation treatment. For blueberry samples, it is possible to use the EPR spectrum of Mn^{2+} ions as an internal standard to prove irradiation but this approach is not laid down in the protocols of the European Committee for Standardization. Protocol EN 13708 for irradiation foods, containing crystalline sugar is not applicable for the investigated samples.

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