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NATURAL AND GAMMA INDUCED FREE RADICALS IN DRIED FRUITS. AN EPR STUDY

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

Abstract

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The aim of the present study is to prove radiation processing in air dehydrated dates, prunes and figs, as well as to recommend which part of the fruit to sample. The detection method is Electron Paramagnetic Resonance (EPR) spectroscopy. Before irradiation in date stone, prune stone and flesh, and fig seeds EPR spectroscopy was detected a weak singlet line, whereas date flesh and fig flesh are EPR silent. After gamma-irradiation in date stone and flesh, and fig flesh a “sugar-like” EPR spectrum is recorded. In prune stone a typical “cellulose-like” spectrum is detected, whereas in prune flesh and fig stone only singlet line is recorded as before radiation treatment. Of research done it can be seen that the irradiation is proved in a different part of the fruit. European Protocol EN 13708 is applicable to irradiated dried dates and figs, whereas Protocol EN 1787 for dried prunes.

Key words: dried fruits, gamma-irradiation, EPR spectroscopy

Introduction

Food irradiation is introduced mainly to reduce spoilage losses and to improve hygienic quality. Electron Paramagnetic Resonance (EPR) is one of the most promising methods for identification of irradiated foodstuffs. Three protocols using EPR technique were adopted by the European Committee for Standardization. The first protocol is relative to meat and fish bones (EN 1786), the second one to food containing cellulose, such as pistachio nuts, berries, dry herbs and spices (EN 1787). The third one was voted later, relative to food containing crystalline sugars, such as dried figs, mangoes, papayas and raisins (EN 13708). Identification procedure of dry herbs and spices is based on the fact that, whereas non–irradiated samples exhibit only one weak singlet EPR signal, its amplitude significantly increases after irradiation together with the appearance of two weak satellite lines. This pair of lines is believed to due to free radicals of cellulose generated by gamma–irradiation and only their presence is considered in the Protocol (EN 1787) as unambiguous evidence for previous radiation treatment of plants. This so called “cellulose-like” EPR spectrum is also detected in seeds, stalks and stones of fruits (Raffi and Agnel, 1989; Stachowicz et al., 1996).

Drying removes most of the water contained in the fresh fruit. This interfere the growth of microorganisms - bacteria, yeast, fungi, that cause decay and deterioration of the fruit. On the other hand the reduction of the amount of water leads to an increase of the concentration of sugars and acids, which have a preservative effect (Doufi et al., 1998). There are three different methods for dehydration of fruits – by air, lyophilization and by osmosis. Dried fruits are rich in sugars. The amount ranges from 60 to 75%, the main components are D-fructose, D-glucose and D-sucrose. After irradiation of dried fruits a complex signal was observed. It has a width of 6-8 mT, centered in approximately g = 2.004 and due to the para-

**Corresponding author:* iliana_nacheva@abv.bg

magnetic centers, generated in the sugar crystals. Since these radicals are attached in the crystal lattice, the possibility of recombination is low, and therefore they are very stable over time. In the food can present different types and in different proportions mono- and di-saccharides. As a consequence may register various spectra of the same kind, it depends on the type, structure and crystallinity of the radicals of the mono- and di-saccharides present in the sample (Helle and Linke, 1992; Esteves et al., 1999). Therefore, the registered EPR spectrum after irradiation is called "sugar-like" as the various mono- and di-saccharides are present in different amounts in the sample as a result may dominate different lines in the EPR spectrum (Yordanov and Georgieva, 2004).

The purpose of the present study is to investigate the air-dried dates, figs and prunes and compared the radiation-induced signals in different parts of the fruit in order to prove irradiation.

Materials and Methods

Food samples and irradiation

All samples for investigation (dates, figs and prunes) are purchased from local markets. Two sets are prepared of each sample and kept in polyethylene bags – one irradiated and the other remaining non-irradiated for control.

All samples are irradiated by "Gamma 1300" irradiator (^{137}Cs, dose rate 0.42 kGy/h). The irradiation is performed at room temperature in the air.

Instrumentation

All EPR measurements were performed at room temperature on Bruker ER 200 D SRC spectrometer operated in X–band. Standard rectangular cavity (ER4102ST) operating in TE_{102} mode was used. The g-values of all samples were estimated using "EPR marker" available in the F-F Lock module (ER 033) – in our spectrometer g-mark is 2.0050.

Results and Discussion

EPR spectra before irradiation

Before irradiation in date stone, prune stone and flesh, and fig seeds EPR spectroscopy was detected a weak singlet line with g factor 2.0048, which is equal to that observed in skin, stone or seed samples of fresh fruits. It is attributed to free radicals of semi-quinones (Swartz et al., 1972), lignin (Maloney et al., 1992) or to oxidation products of fatty acids present in some fruits and vegetables (Ikeya et al., 1989). Date and fig flesh are EPR silent.

EPR spectra after irradiation

According to protocol EN 13708 in air dehydrated fruits after irradiation must be registered multicomponent "sugar-like" EPR spectrum, which is positive proof that the sample was subjected to radiation treatment. At the same time in air dehydrated fruits do not always detect the typical "sugar-like" spectrum, and in various types of fruit such as figs and dates that contain little sucrose, and more glucose and fructose EPR spectra after irradiation can have the different structure.

After irradiation date stone (Figure 1a) and flesh (Figure 1b) exhibit typical "sugar-like" EPR spectra. It should be noted that these two spectra are different due to the different content of the saccharides in the date stone and flesh. Therefore different lines from sugar crystals dominate in the registered EPR spectra. In dehydrated by osmosis fruits investigated in our laboratory previously (Yordanov and Aleksieva, 2007) also were registered "sugar-like" EPR spectra, that is why European standard is fully applicable. Protocol EN 13708 is certified for air dehydrated raisins, papaya, mango and figs and is recommended sampling "outer portion" of the fruits. The results show that a typical multi-component spectrum is recorded

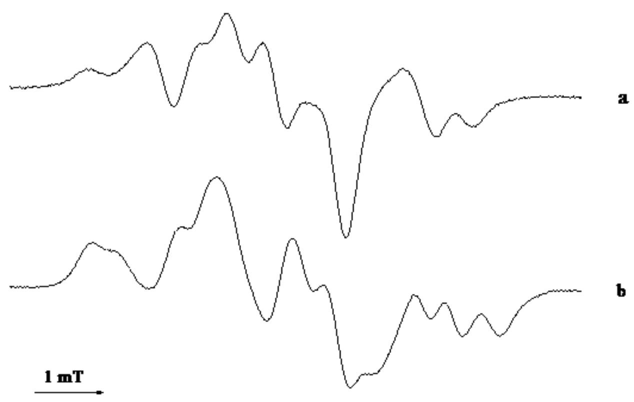

Fig. 1. EPR spectra of gamma-irradiated date stone (*a*) and flesh (*b*)

after the radiation not only in the sample from the flesh of a date, but also in stone. Up to now stones of dates are the only one stones from fruit in which "sugar-like" EPR spectrum can be detected.

On irradiation with gamma rays were subjected stones (Figure 2a) and flesh (Figure 2b) of dehydrated on the air prunes. After irradiation in prunes stone was detected characteristic "cellulose-like" EPR spectrum, which was observed in stone of fresh prunes (Raffi and Agnel, 1989) and also recently from our laboratory in irradiated flesh of fresh fruits (Yordanov and Aleksieva, 2009) and lyophilized aronia (Nacheva et al., 2013). For irradiated with 1 kGy stones "cellulose-like" spectrum is less intense. In contrast, in irradiated with 3 kGy stones, satellite lines (marked with arrows in Figure 2a) are more intense. The fleshy parts of the prunes before and after irradiation showed no difference in the signal nor to the g-factor, nor significant change in the intensity of the registered singlet line (Figure 2b). The lack of difference in the signals before and after irradiation makes it impossible to identify

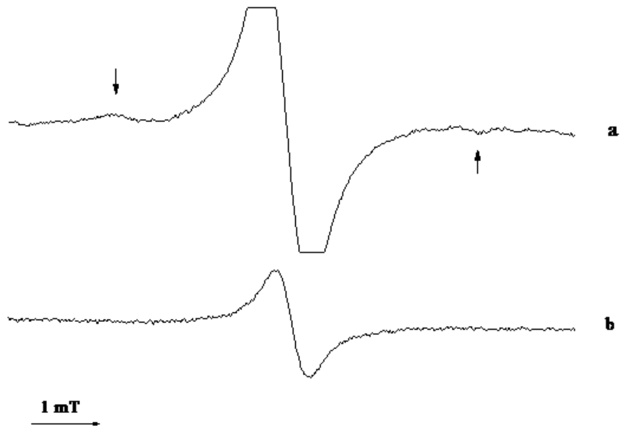

Fig. 2. EPR spectra of gamma-irradiated prune stone (*a*) and flesh (*b*)

as irradiated the flesh of prunes, dehydrated in air. Such a result is received for freeze-dried prunes (Yordanov et al., 2006), in which is registered only a singlet, but whose g-factor and intensity significant change after radiation treatment.

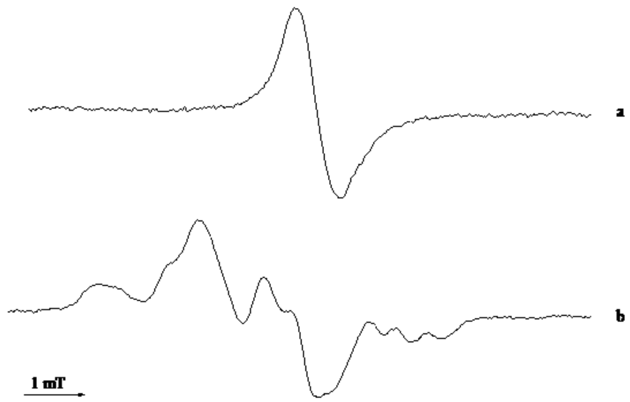

Fig. 3. EPR spectra of gamma-irradiated fig stone (*a*) and flesh (*b*)

Irradiated with 1 kGy seeds of figs show only expansion of the singlet signal compared to that recorded before irradiation. By subjecting the radiation treatment with a dose of 3 kGy was not detected the satellite lines of cellulose (Figure 3a). The registered spectrum of seeds represents a relatively weak singlet, wherein the specific signal is not observed. Under the influence of gamma irradiation in saccharides of flesh is generated typical multicomponent EPR spectrum (Figure 3b). In this kind of "sugar-like" spectrum dominated lines of glucose.

Conclusion

Detection of "sugar-like" EPR spectra at the date, regardless of which of the saccharides has the largest contribution to the EPR spectrum, or which part of the fruit is subjected to analysis, is sure evidence of radiation treatment. "Cellulose-like" EPR spectrum is specific for irradiated samples of prune stone and proves irradiation. Therefore, to identify a previous irradiation in air dehydrated prunes is recommended sampling stone. Identification a previous irradiation in air dehydrated figs recommended sampling for analysis of flesh, because "sugar-like" EPR spectrum is recorded. Protocol EN 13708 is applicable to irradiated dried dates and figs, whereas Protocol EN 1787 for dried prunes.

References

Doufi, L., J. Raffi and P. Stocker, 1998. A point about electron paramagnetic resonance detection of irradiated foodstuffs. *Spectrochim*. Acta Part A, **54**: 2403-2412.

EN 1786, 1997. Detection of Irradiated Food Containing Bone: Analysis by Electron Paramagnetic Resonance, *European Committee for Standartization*, Brussels.

EN 1787, 2000. Detection of irradiated food containing cellulose, method by ESR spectroscopy. *European Committee for Standardization*, Brussels.

EN 13708, 2001. Detection of irradiated food containing crystalline sugar by ESR spectroscopy. *European Committee of Standardization*, Brussels.

Esteves, M. P., M. E. Andrade and J. Empis, 1999. Detection of prior irradiation of dried fruits by electron spin resonance (ESR). *Rad. Phys. Chem*., **55**: 737-742

Helle, N. and B. Linke, 1992. ESR for Detecting Gamma-Irradiated Foodstuffs. *Bruker Report,* **91/92**, 8-9.

Ikeya, M., O. F. Baffa and S. Mascarenhas, 1989. Quality assessment of coffee beans with ESR and gamma-irradiation. *Appl. Radiat. Isotopes*, **40:** 1219-1222.

Maloney, D. R., B. J. Tabner and V. A. Tabner, 1992. An electron spin resonance study of gamma-irradiated fruits. *Radiat. Phys. Chem*., **39**: 309-314.

Nacheva, I., K. Aleksieva, K. Dimov, D. Miteva and Tsv. Tsetkov, 2013. Identification of gamma irradiated lyophilized forest fruits by EPR spectroscopy. *Bulg. J. Agricult. Sci*., **19**: 293-297.

Raffi, J. J. and J. P. L. Agnel, 1989. Electron Spin Resonance Identification of Irradiated Fruits. *Radiat. Phys. Chem*., **34**: 891-894.

Stachowicz, W., G. Burlinska, J. Michalik, A. Goclawska and K. Ostrowski, 1996. EPR spectroscopy for the detection of foods treated with ionizing radiation. In: Detection Methods of Irradiated Foods, *The Royal Society of Chemistry*, pp. 23-32.

Swartz, H. M., J. R. Bolton and D. C. Borg (eds.), 1972. Biological Applications of Electron Spin Resonance. *Wiley,* New York.

Yordanov, N. D. and K. Aleksieva, 2007. EPR study on gamma-irradiated fruits dehydrated via osmosis. *Radiat. Phys. Chem.,* **76**: 1084-1086.

Yordanov, N. D. and K. Aleksieva, 2009. Preparation and applicability of fresh fruit samples for identification of radiation treatment by EPR. *Radiat. Phys. Chem*., **78:** 213-216.

Yordanov, N. D. and E. Georgieva, 2004. EPR and UV spectral study of gamma- irradiated white and burned sugar, fructose and glucose. *Spectrochim. Acta A*., **60**: 1307-1314.

Yordanov, N. D., K. Aleksieva, A. Dimitrova, L. Georgieva and E. Tzvetkova, 2006. Multifrequency EPR study on freeze-dried fruits before and after X-ray irradiation. *Radiat. Phys. Chem*., **75**: 1069-1074.

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