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Gamma-irradiated dry fruits An example of a wide variety of long-time dependent EPR spectra

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Abstract

EPR spectra of dry, sugar containing fruits—raisins, sultanas, figs, dates, peaches, blue plums and chokeberry recorded before and after irradiation with gamma-rays, are reported. It is shown that weak singlet EPR line with 2.0031 ± 0.0005 can be recorded before irradiation of seeds, stones or skin of chokeberry, figs and raisins as well as flesh of blue plum, raisins and peaches. EPR signals of various shape are distinguished after irradiation in different parts of the fruits, as well as in randomly cut pieces of them:

- Seeds of raisins, chokeberry and figs give a singlet line. Stones from blue plums and peaches exhibit typical "cellulose-like" EPR signal consisting of an intense singlet line with $g = 2.0033 \pm 0.0005$ and 2 week satellite lines situated ca. 30 G left and right to it. Stones of dates are the only sample in which "sugar-like" spectrum is recorded.
- Skin of raisins and figs exhibits "sugar-like" EPR spectrum whereas that of dates and chokeberry—a singlet line. Under the same experimental conditions skin of sultanas, peaches and blue plums are EPR silent.
- Flesh of raisins, sultanas, figs, dates and peaches exhibits "sugar-like" EPR spectrum, flesh of blue plums gives a singlet EPR line and that of chokeberry is EPR silent.

As a result, randomly cut pieces of dry fruits suitable for EPR studies, containing various constituents, exhibit different in shape and intensity EPR spectra. Kinetic studies followed for 1 year on the time stability of all reported EPR signals indicate that intensity ratio between the simultaneously appearing EPR signals in particular fruit varies from 1:20 immediately after irradiation to 1:0.5 at the end of the period. These observations open a new possibility for identification of irradiated fruits – using the magnitude of the intensity ratio to find the approximate date of radiation processing in the first ca. 30–100 days.

Keywords: Sugar containing fruits; Irradiation; EPR; Identification

1. Introduction

Irradiation of foodstuffs by high energy is a safe, secure, cheap and clean sterilization procedure recommended and used recently. However, this manipulation yields free radicals in foods, some of them recombining to give new species the impact of which on the living organism is not well studied. Therefore, the control on the radiation procedure has become important and generated many studies in the last two decades. As a result, European Committee of Standardization issued several standards for identification of radiation processing of foodstuffs. Three of them use EPR spectrometry as a detection tool [1–3]. The first one [1], giving unambiguous conclusions, is devoted to identification of bones containing meat. The second [2] refers to the discovery of radiation treatment of cellulose containing foods. In this case, radiation processing only is unambiguously identified if "cellulose-like" EPR spectrum appears and the results obtained following this standard are sometimes under question. Many studies were recently performed to improve this standard (see, for example, [4–7]). The last (and new) protocol EN13708 [3] treats the identification of radiation processing of crystalline sugar containing foods. It is stated in it that a multicomponent "sugar-like" EPR spectrum should be recorded after irradiation due to different type and composition of mono-

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and di-saccharides. Protocol [3] is only validated for dried figs, mangoes, papayas and raisin. However, the statements in it are in contradiction with the results for some other dry fruits obtained even before its issuing [8,9]. Therefore, in order to extend the number of dried fruits for which previous radiation processing can be unambiguously identified, in the present paper, we report our results on the EPR studies of dry fruits or randomly cut pieces from them, as well as their components (seeds or stones, flesh and skin) before and after gamma-radiation processing. Three different types of EPR spectra—"sugar-like", "cellulose-like" and a singlet line were recorded in it. Moreover, even for some of the fruits considered and given as examples in [3], the shape of their EPR spectra depends of the time passed between irradiation and investigation.

2. Experimental

Dry raisins, sultanas, figs, dates, peaches and blue plums were purchased from the local market. Some of them were in closed packed form, other were open in the air. Third (blue plums, chokeberry and peaches) were home dried.

3. Preliminary studies of the received material

Preliminar test for water content was immediately applied after receiving the materials for investigation and specially those obtained open in the air. For that purpose, the sample was weighed, transferred to a standard laboratory oven and kept at 30 °C for 8 h in it. After that the sample was weighed again. Loss of 5–8% of weight is due to the humidity of dry fruit. More than 10% difference between the weightings suggests higher water content (or moisture over the ambient) in the investigated samples (30–40% loss in the weight was recorded in some rare cases of our practice!). Such samples are not suitable for further studies due to the increased rate of recombination of radiation-induced free radicals making questionable the correct conclusion based on EPR investigations. Such losses in the weight were not recorded for fruits in packed form.

4. Processing and irradiation of the samples

Samples selected for further studies were divided into several groups each containing randomly cut pieces of the full fruit suitable for inserting in the EPR sample tubes, as well as seeds or stones, flesh and skin only. Each sample was accommodated in closed polyethylene bag and transferred for irradiation.

The irradiation was realized with ^{60}Co source at the dose rate of 360 Gy/h. All samples were irradiated with doses of 10 kGy.

5. Instrumentation

EPR spectra were recorded on a Bruker ER 200D SRC spectrometer operating in X-band. The g-values of all samples were estimated using "EPR marker" available in the F–F Lock module (ER 033) of the spectrometer calibrated in advance by DPPH [10]. For obtaining more precise results each sample of skin, flesh or part of the whole fruit was accommodated in a separate both sides open EPR sample tube (i.d. 5 mm, o.d. 6 mm) facilitating the cleaning after use. A cotton tampon was inserted in the lower side of the sample tube and the material under study was situated over it. Sample tubes were always positioned exactly in the EPR cavity centrum. Kinetic studies on the decay of the radiation-induced EPR signals were performed for a period of 1 year versus Mn^{2+}/MgO reference material permanently installed in the EPR cavity as described before [11]. Each data point was taken as the average of at least five independent measurements.

6. Results and discussion

Flesh of all studied samples was EPR silent before irradiation with the exception of blue plums, raisins and peaches the flesh of which exhibits a weak singlet signal with $g = 2.0031 \pm 0.0003$. On the other hand, seeds, stones and skin of all other dry fruits studied exhibit weak singlet signal with g-factor varying for different fruit in the frame of 2.0031 ± 0.0005 (with the exception of figs, the skin of which is EPR silent). Similar singlet EPR line was recorded by other authors for achens of fresh strawberry [9,12,13], grapes [9,14,15], cherry [9] and pear [16].

EPR signals of big variety of shape and intensity were recorded after irradiation depending on the sample and/or its components which will be separately considered. Since the intensities of the EPR signals are time dependent in a different way, and the samples of dry fruit, randomly cut into pieces suitable for EPR studies, contain different components, they can be expected to exhibit different in shape EPR spectra due to the prevailing effect of one or other component's peculiarities. These observations justify the kinetic studies on the EPR decay not only in individual components of the fruit, but also in randomly cut pieces of fruits.

6.1. Seeds or stones

After irradiation seeds of raisins, figs and chokeberry exhibit singlet EPR signal (Fig. 1) with $g = 2.0033 \pm 0.0005$ with considerably increased intensity compared to non-irradiated fruits. The singlet signal cannot be used for identification of

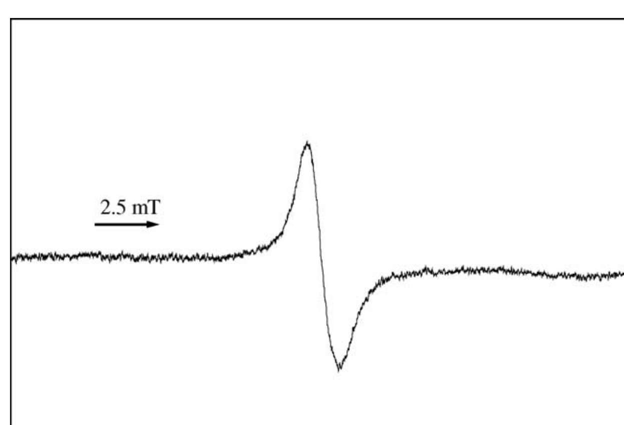

Fig. 1. Typical singlet EPR line recorded before irradiation in seeds, stones and skins of the studied dry fruits as well as in some irradiated parts of them (see the text).

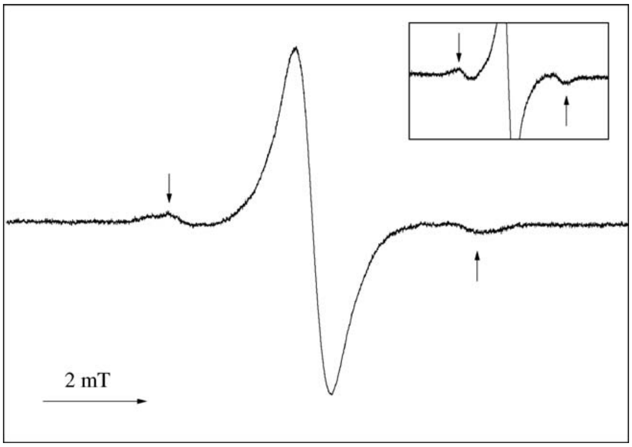

Fig. 2. Typical "cellulose-like" EPR spectrum recorded in some parts of irradiated (see the text). *Inset:* The same spectrum recorded with 10 times increased gain.

previous radiation treatment because its intensity cannot be specified. After irradiation stones of blue plum and peaches exhibit "cellulose-like" EPR signal characterized [13,17] with an intense singlet EPR line with $g = 2.0033 \pm 0.0005$ and 2 week satellite lines situated ca. 30 G left and right to it (Fig. 2). Contrary, after irradiation stones of dates exhibit a "sugar-like" EPR spectrum (Fig. 3). Therefore, EPR spectra of stones of apricots, plums, peaches and dates permit unambiguous identification of previous radiation processing [17,20].

6.2. Skin of fruits

After irradiation skin of raisins and figs exhibits "sugar-like" EPR spectrum [3] whereas that of dates and chokeberry—a singlet line. The presence of "sugar-like" EPR spectrum in the skin of raisins and figs can be considered as unambiguous evidence of radiation processing of the fruits. The *g*-factors of the singlet EPR line of dates and chokeberry are not changed during irradiation. The difference is only in the peak-to-peak intensity of the singlets, which are significantly increased in irradiated samples. As noted before, singlet lines recorded in skin of irradiated dates and chokeberry, cannot be used for identification of previous radiation treatment due to the fact that EPR spectrometry is not calibrated [18,19] and the signal intensities cannot be specified. Skin of sultanas, peaches and blue plums is EPR silent.

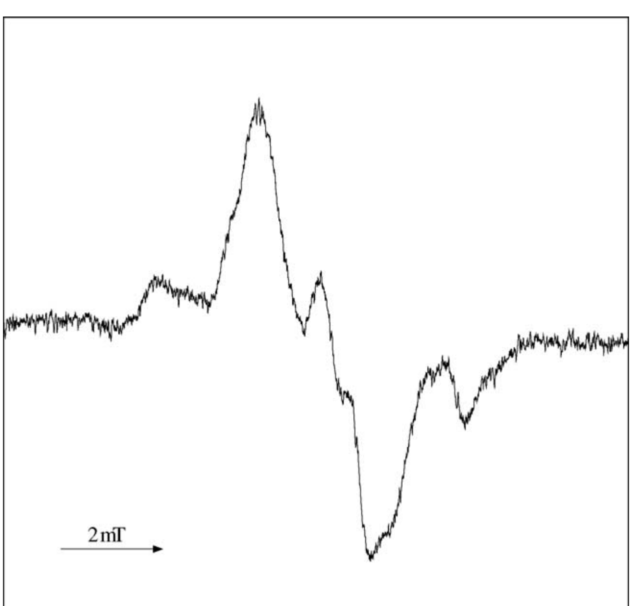

Fig. 3. Typical "sugar-like" EPR spectrum recorded in irradiated dry fruits (for details see the text).

6.3. Flesh of studied fruit samples

After irradiation flesh of dry raisins, sultanas, figs and dates exhibits "sugar-like" EPR spectrum. Under the same experimental conditions, flesh of blue plums only exhibits a singlet EPR line, whereas chokeberry flesh is EPR silent. Thus, in accordance with [3] the presence of "sugar-like" EPR spectrum can be considered as unambiguous evidence of previous radiation treatment of the studied dry fruits. On the other hand, the absence of "sugar-like" EPR spectrum or the only presence of singlet EPR line can not be considered as unambiguous evidence that sample was irradiated. Similarly to the observations of other authors [21,22] EPR spectrum was not observed in irradiated flesh of peaches.

6.4. Samples of the whole fruit or its randomly cut pieces

Because most of the studied dry fruits were large enough to be accommodated in the EPR sample tube, parts of them were randomly cut into pieces suitable for EPR investigations. These parts were considered as representative for the whole fruit. Flesh, skin and seeds were studied as a mixture in this procedure.

Samples of whole raisins, sultanas and peaches exhibit "sugar-like" EPR spectrum (Fig. 3) and according to the studies of their separate components it is due to the flesh. On the other hand, EPR spectra of whole chokeberry and dates are singlets, which may be attributed to seeds and skin of chokeberry or skin of dates. The most intriguing is the EPR spectrum of figs given on Fig. 4a. It is a "cellulose-like" spectrum in shape (Fig. 2), but is completely different from it. (Similar EPR spectrum was previously reported for juniper berries [23].) The spectrum is also different from that given as an example (Fig. 4b) in protocol EN13708 [3] for "sugar-like" EPR spectrum of irradiated figs.

Stones, if available, were neglected in the considerations mentioned above, while the present studies show that the most unambiguous evidences of radiation processing of dates, apricots and blue plums may be obtained by investigation on parts of their stones (if available). In these cases, "sugar-like" or "cellulose-like" EPR spectrum is observed in radiation processed samples.

6.5. Kinetic studies on the time stability of the reported EPR signals

In order to find the time stability and the changes in the radiation-induced EPR signals of all reported parts of the fruits,

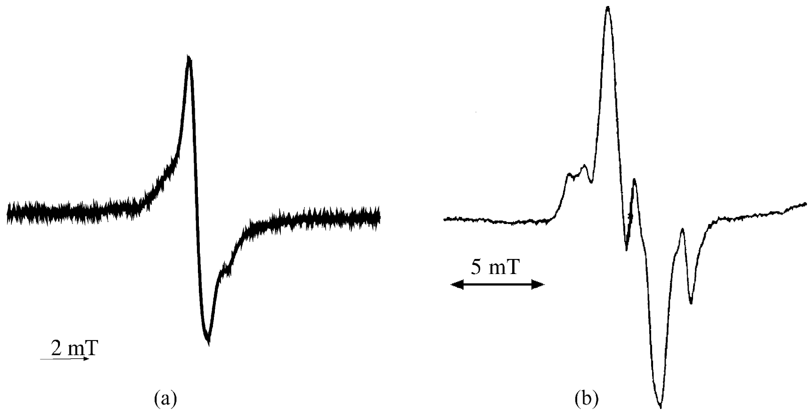

Fig. 4. EPR spectrum of gamma-irradiated peace of figs: (a) this work, (b) following protocol EN13708.

the studies were extended for a period of 1 year. It was found that radiation-induced "cellulose-like" EPR signals in stones of blue plums and peaches decay to ca. 50% within 30–40 days after irradiation, but they are enough strong to be recorded more than 1 year period [17]. The intensity of the initial (before irradiation) singlet signal in seeds is ca. 10–20 times increased after radiation processing and decays to 50% within 20–30 days after that (Fig. 5). It was also found that the intensity ratio between different radiation-induced EPR signals in particular fruit varies from 1:20 immediately after irradiation to 1:0.5 at the end of the period. Therefore, randomly cut pieces of dry fruits, taken for investigation, containing different constituents exhibit different in shape EPR spectra due to the prevailing effect of one or other component's peculiarities.

Kinetic studies can explain the shape of the EPR spectrum of randomly cut pieces of irradiated figs (Fig. 4a) recorded 1 month after radiation processing. Contrary to protocol EN 13708 [3] expecting "sugar-like" spectrum (Fig. 4b) the spectrum in Fig. 4a is an intense singlet line with two sets of weak wings situated ca. 12 and 30 G left and right of it. Having in mind the kinetic behavior of the separate parts of figs, the EPR spectrum of the whole fruit (Fig. 4a) may be suggested as a superposition of two spectra (Fig. 6a)—the intense singlet line coming from the seeds and two pairs of wings: one coming from two intense lines

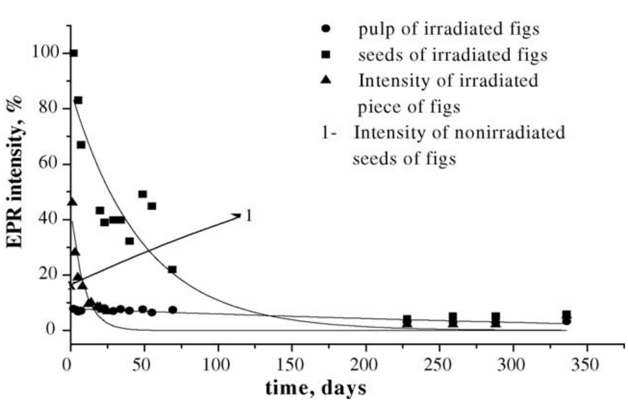

Fig. 5. Decreasing intensities of the EPR spectra of different parts of dry figs after irradiation. With (x) on the ordinate axis is marked the intensity of non-irradiated sample.

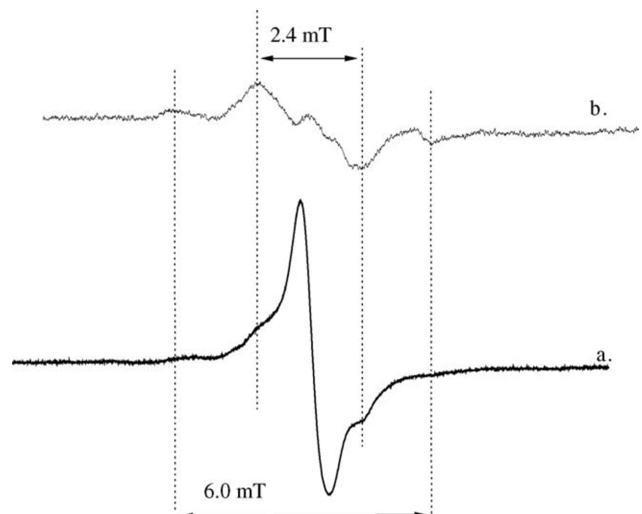

Fig. 6. Comparison of EPR spectrum taken from gamma-irradiated randomly cut piece of figs (a) with a "sugar-like" spectrum recorded from the flesh (b).

situated at ca. 24 G to each other and two other less intense lines situated ca. 60 G to each other coming from the weak "sugar-like" spectrum (Fig. 6b) of flesh. This finding opens a new not exploited up to now possibility for identification of irradiated fruits—dating the irradiation procedure. Specially for figs dating is possible within the first ca. 30–100 days after radiation processing.

7. Conclusions

The present studies on EPR spectra of dry sugar containing fruits—raisins, sultanas, figs, dates, peaches, blue plums and chokeberry recorded before irradiation with gamma-rays show that some of them are EPR silent or exhibit a weak singlet EPR line with 2.0031 ± 0.0005 in seeds or stones and some of them in skin and flesh. After irradiation, certain parts of the fruit or randomly cut pieces from them show the following different in shape and intensity EPR signals:

- Seeds of raisins, chokeberry and figs give a singlet line. Stones from blue plums and peaches exhibit typical "cellulose-

like" EPR signal consisting of an intense singlet line with $g = 2.0033 \pm 0.0005$ and 2 week satellite lines situated ca. 30 G left and right to it. Stones of dates are the only samples in which "sugar-like" spectrum is recorded.
- Skin of raisins and figs exhibits "sugar-like" EPR spectrum whereas that of dates and chokeberry—a singlet line. Under the same experimental conditions skin of sultanas, peaches and blue plums is EPR silent.
- Flesh of raisins, sultanas, figs, dates, apricots and peaches exhibits "sugar-like" EPR spectrum, blue plums flesh gives a singlet EPR line and that of chokeberry is EPR silent.

Therefore, the appearance of "sugar-like" or "cellulose-like" EPR spectra during the investigation of a dry fruit is an indication of previous radiation processing. The presence of singlet EPR line cannot be considered as indication for radiation processing since its intensity cannot be specified and other approaches [4–6] are more promising for this purpose.

Kinetic studies on the decay of EPR intensities show that signal intensity ratio varies from 1:20 immediately after the irradiation to 1:0.5 within 1 year period with different decay rate. This fact justifies the appearance of different in shape EPR spectra of randomly cut pieces from the fruit containing different constituents as due to the prevailing effect of one or other component's peculiarities. On the other hand, it opens a new possibility to identify irradiated fruits and to find the date of irradiation.

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