Effect of Irradiation Dose, Microwave Power, and Storage Time on the Free Radical Concentration in γ-Irradiated Black Olive (Olea Europaea) Seeds

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Abstract

Dosimetric properties of irradiated black olive seeds cultivated in Turkey were investigated through Electron Paramagnetic Resonance (EPR) technique. In unirradiated samples singlet EPR signals which are results of polyphenol oxidation in plants were obtained. Two satellite peaks on the sides of the central singlet signal attributed to cellulose radicals were observed after the samples were irradiated up to 10 kGy. In order to describe the variation of EPR signal intensity with absorbed radiation dose, several mathematical equations were tried. Moreover time dependency of the intensity of the central EPR signal of the irradiated sample was analyzed to study the stability of the free radicals. Based on the results of the study, it can be concluded that EPR technique can be used to identify unirradiated and irradiated black olive seeds even after two months.

Keywords: EPR; Food Irradiation; Absorbed dose.

1. Introduction

Table olives are a traditional component of the Mediterranean diet and are largely consumed in the world [1]. Turkey has an important place in the world’s olive production, having a 7% share of total olive and 17% of total table olive production [2]. Treatment of food by specific ionizing radiations to improve microbiological safety and storability is one of the most extensively studied technology of the 20th century [3]. Ionizing radiation has been widely used to sterilize both foods and drugs [4-9]. In this context, it is important to find methods distinguishing between irradiated and
unirradiated foods and drugs. Unlike alternative methods, controlled exposure to gamma radiation kills microorganisms in a single procedure that does not involve a quarantine period [10]. Since using radiation treatment is prohibited or limited in several countries, it is important to control irradiation. To control this, analytical methods such as thermoluminescence or chemiluminescence have been used to distinguish irradiated from unirradiated foods. In this context, electron paramagnetic resonance (EPR) spectroscopy is one of the established methods for identifying irradiated food [11-13]. In addition, EPR technique is simple, specific and rapid for detecting radiation processed foods [14]. What is more, European Committee of Normalization (CEN) has released EN 1787 standards for detecting irradiated foods by EPR spectroscopy for foods containing cellulose [15]. Unirradiated samples of dry spices and some plants exhibit only one weak singlet EPR signal. Irradiation increases the intensity of singlet signal, and forms two weak satellite peaks [16]. The aim of this study is to find out whether black olive seeds have been irradiated with ionizing radiation through EPR technique. In this paper, the variation of EPR signal intensity of the irradiated black olive seeds versus microwave power (mW), irradiation dose (kGy), and storage time (days) was investigated.

2. Experimental

Seeds of black olives cultivated in Marmara region, Turkey were used in this paper. The seeds of olives were scraped clean of soft tissue and powdered in a grinder. Almost the same weight of sample (≈30 mg) for each irradiation dose was placed in EPR quartz tubes. Powder of black olive seeds was irradiated at 0.5, 1, 1.5, 3, 7, 10, 12 and 15 kGy by a $^{60}$Co-$\gamma$ ray source at room temperature. After the irradiation, all samples were kept in plastic bags, and left in the dark at room temperature. EPR measurements of all samples were taken 2 days after irradiation to avoid any short-lived paramagnetic species. The EPR spectra of both irradiated and non-irradiated powder of black olive seeds were recorded at room temperature with a bruker EMX model spectrometer operating at microwave power 0.499 mW, microwave frequency of 9.8 GHz, modulation amplitude 0.104 mT, magnetic field modulation frequency 86 kHz. The g factors were calibrated by comparison with a DPPH sample ($g=2.0036$).

3. Results and discussion

3.1 EPR spectra of unirradiated and irradiated black olive seeds

EPR spectrum of unirradiated black olive seeds recorded at room temperature were given in figure 1. The EPR spectrum of unirradiated sample exhibits a sharp and clear singlet EPR signal centered at 2.0088 without any hyperfine structures. This g value obtained compares well with those reported in the literature [17-19]. The origin of this singlet line is not clear. However, the signal observed before irradiation can be attributed to semiquinone radicals produced by oxidation of polyphenols naturally present in plants [20-22]. The EPR spectra of both irradiated and non-irradiated powder of black olive seeds were recorded at room temperature with a bruker EMX model spectrometer operating at microwave power 0.499 mW, microwave frequency of 9.8 GHz, modulation amplitude 0.104 mT, magnetic field modulation frequency 86 kHz. The g factors were calibrated by comparison with a DPPH sample ($g=2.0036$).
radiation treatment [27]. EPR signals of the irradiated black olive seeds are distinguishable from the signals of unirradiated sample.

Firstly, the intensity of central singlet EPR signal of both unirradiated and irradiated samples as a function of the microwave power was investigated in the range of 0.1-20 mW at room temperature. While the curve belongs to unirradiated sample starts to saturate almost at 3 mW, 10 kGy irradiated sample saturates at almost 4 mW (Figure 4). As it is seen from the figure, microwave saturation features of unirradiated and irradiated samples are different from each other. These results also indicate that the origin of the radicals present in unirradiated and irradiated samples is different.

3.2 Dose-response curve

Aside from qualitative detection, EPR can be used for dose estimation [28]. Therefore, the effect of increasing radiation dose on the spectra of olive seeds was studied. Samples of black olive seeds irradiated in the dose range of 0.5-15 kGy were used to construct dose-response curve. Variation of peak-to-peak central singlet signal intensity with the applied radiation dose is presented in Figure 5. It is seen, the experimental and calculated data was found to agree well with each other. The g-factor and line widths do not change in the studied dose range. It is observed that the intensity of the central EPR signal increases gradually with the increase of irradiation dose. Several mathematical functions were tried to describe evolution of the central EPR signal with absorbed radiation dose. Linear, exponential and polynomial functions are frequently used for this purpose [29-31]. In these functions, I and D are used for EPR signal intensity and treated irradiation dose in kGy, respectively and other parameters are constants to be determined. If the values calculated from these mathematical functions together with correlation coefficients are taken into consideration, the dose response curve of olive sample is explained best by polynomial function \((r^2=0.9979)\). It should be noted that no attempt has been made to force the regression through zero. The numerical results of the fitting are presented in Table 1. Dose-response curve of the irradiated sample is seen to be quite compatible with linear, exponential and polynomial functions.

3.3 Radical decays at room temperature

Tests were carried out to investigate whether storage had an effect on the free radicals concentration [32]. The powder of black olive seeds irradiated at a dose of 10 kGy was studied to determine the effect of storage on the signal intensity of radiation-induced free radicals. Samples were kept in the dark at room temperature over a period of 60 days, the EPR spectra were recorded periodically during this storage time and results are shown in figure 6. It can be clearly seen that the signal intensity of the main central signal decreases rapidly in the first 10 days then it decreases more slowly. As it is seen, the decline in the signal intensity is about 29% during the first 10-day period and about 73% over a 60-day period. From the experimental spectrums recorded 26 days after irradiation, no satellite peak was detected. The results indicate that EPR spectroscopy could be used to distinguish irradiated black olive seeds from non-irradiated ones during the storage time.

4. Conclusions

Non-irradiated samples of black olive seeds represent a singlet EPR signal. After irradiation of the samples with gamma rays up to 10 kGy, two satellite peaks, with a separation of 6 mT, at the left and right of the main EPR signal were observed. The presence of these satellite peaks can be used as evidence of irradiation treatment. Irradiation up to 10 kGy makes these satellite peaks more visible. In addition, irradiation up to 10 kGy increased the intensity of the main signal without any changes in g-factor. The EPR signal intensity of the main signal was found to depend on the absorbed radiation dose. It can be concluded that, irradiation causes an increase in the amount of free radicals. The powder of black olive seeds irradiated at a dose of 10 kGy exhibited a singlet and
observed to be stable for 60 days. It was also found that samples irradiated at a dose 7 kGy and 10 kGy exhibited different microwave saturation features. These results indicate that the origin of the radicals present in unirradiated and irradiated samples is different.

The analyses clearly revealed that EPR technique easily enables us to identify and distinguish radicals that are naturally present and produced after irradiation with gamma rays in black olive seeds. Additionally, EPR measurements are non-destructive and require minimal time for sample preparation. However, further studies are needed to identify radiation sensitivity of foodstuffs. The investigation of radiation sensitivity of the black olive seeds can be helpful for similar studies.

References


Figure Captions

Figure 1. EPR spectrum of unirradiated black olive seeds recorded at room temperature.

Figure 2. (a) EPR spectrum of 7 kGy irradiated black olive seeds recorded at room temperature. (b) EPR spectrum of 10 kGy irradiated black olive seeds recorded at room temperature.

Figure 3. (a) EPR signal intensity of unirradiated black olive seeds as a function of square root of microwave power.
(b) EPR signal intensity of 10 kGy irradiated black olive seeds as a function of square root of microwave power.

Figure 4. Dose-response curve of black olive seeds.

Figure 5. EPR signal intensity of black olive seeds irradiated at a dose of 10 kGy as a function of time.

Table 1. Mathematical functions calculated for dose-response curve of gamma irradiated black olive seeds.
Fig. 2
Fig. 3

Fig. 4
Fig. 5

Table 1

<table>
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<th>Parameter 1</th>
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