

Detection of Irradiated Wheat Using the Electron Paramagnetic Resonance Spin Probe Technique

DİLEK DADAYLI,^{1,2} M. MARAL SÜNNETÇİOĞLU,³ HAMİT KÖKSEL,⁴ SÜEDA ÇELİK⁴

ABSTRACT

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In this study, the electron paramagnetic resonance (EPR) spin probe technique is applied as a detection method in the differentiation of irradiated and unirradiated wheat seeds. Two wheat cultivars, Kunduru and Bezostaya, were used. Aqueous solutions of 4-hydroxy-TEMPO (TANOL) spin probe prepared with a line-broadening agent, potassium ferricyanide, were used in all experiments. The EPR spectra of the sam-

ples were recorded against time. A decrease in the signal intensity and a change in the shape of the intensity-time curve (rehydration curve) were observed, depending on the applied level of irradiation. The ratio of the lipid and aqueous regions at the high field ($m_1 = -1$) line changes, depending on the dose of irradiation.

Although various preservation methods have been used to prevent food spoilage since ancient times, research to improve food preservation methods is still an important activity in food science and technology. Irradiation is a new approach in this field. The use of ionizing radiation in food technology has been allowed in many countries in recent years. The Joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food stated that irradiation of any food commodity up to an overall average dose of 10 kGy presents no toxicological hazard (Anonymous 1981). The regulations concerning irradiated food show great differences between individual countries. As a part of the regulations for food irradiation, government organizations should be able to detect whether a particular food has been irradiated and measure the irradiation dose. Therefore, in recent years, there has been an increasing interest in developing laboratory methods that allow identification of irradiated food. A number of physical, chemical, and biological measurements have been suggested for the detection of irradiated food in the literature (Dodd et al 1985, Heide and Bögl 1987, Wieser and Regulla 1988, Bögl 1989). Previously, the possibility of using an electron paramagnetic resonance (EPR) technique for the detection of irradiated cereals was investigated. In these studies, it was concluded that EPR technique can be used to detect irradiated samples within a certain period of storage (Onderdelinden and Strackee 1974, Raffi et al 1987, Hunter et al 1988, Troup et al 1989, Köksel et al 1993, Munoz et al 1994).

EPR is a useful tool for investigating physical properties where there is a sufficient concentration of paramagnetic species within a material. However, the difficulty in the application of EPR to most biological systems concerns the low concentration of stable paramagnetic species, which can be overcome by the use of a spin probe technique.

Spin probes are stable paramagnetic species that are highly sensitive to changes in the chemical environment when they are introduced into systems under investigation. They report changes in the chemical environment without disturbing the system. Since the 1960's, the stability and intrinsic magnetic properties of the

nitroxide free radicals have become the most widely used spin probes (McConnell and McFarland 1970, Berliner 1976, Aksyonov and Golovina 1986, Smirnov et al 1988). 4-Hydroxy-TEMPO (TANOL) is a commonly used spin probe (Fig. 1) because of its good water solubility (Kutscher et al 1986). Various line-broadening agents, such as potassium ferricyanide ($K_3Fe(CN)_6$) are used to distinguish the signals of the spin probe from intra- and extracellular regions of biological samples (Eaton and Eaton 1978, Zagnoiko et al 1993, Burchfield et al 1994).

The EPR signal of TANOL and water consists of three well-resolved lines ($m_1 = 1, 0, -1$) as a result of interaction of electron with the nitrogen nucleus. Potassium ferricyanide converts these lines into very broad lines due to exchange interaction (Eaton and Eaton 1978). The membranes of the living cells are impermeable to the line-broadening material. Therefore, after inserting the embryo into the solution prepared with line-broadening material, an unbroadened signal is obtained from the aqueous and lipid parts of the living cells. This signal is superimposed on the broadened signal coming from the solution that surrounds the embryo. Following the insertion of dry embryo in this solution, rehydration begins for the embryo. The penetration of TANOL into the cells can be followed during this rehydration process.

In recent studies, the viability of wheat seeds were investigated by the use of the spin probe technique. Both newly harvested and aged seeds were used in these studies. The correlation between the loss of seed viability and external membrane integrity was monitored using spin probes and a lipid spin label (Smirnov et al 1992; Golovina and Tikhonov 1994; Sünnetçioğlu et al, in press). To our knowledge, this technique has not been applied to the detection of irradiated wheat seeds.

The main objective of this study was to examine the possibility of using spin probe technique for the detection of gamma irradiated wheat.

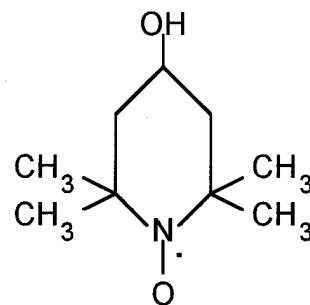


Fig. 1. Nitroxide spin probe 4-hydroxy-TEMPO (TANOL).

¹Department of Physics, Zonguldak Karaelmas University, 67100, Zonguldak, Turkey.

²Corresponding author. E-mail: dadayli@elmas.bim.karaelmas.edu.tr Fax: 01090 03722572070.

³Department of Physics Engineering, Hacettepe University, 06532, Beytepe, Ankara, Turkey.

⁴Department of Food Engineering, Hacettepe University, 06532, Beytepe, Ankara, Turkey.

MATERIALS AND METHODS

One bread wheat sample (cv. Bezostaya) and one durum wheat sample (cv. Kunduru-1149) used in this study were obtained from Field Crops Improvement Center, Ankara, Turkey. Both cultivars were harvested in 1994. The wheat samples were cleaned on a Carter Dockage Tester, placed in polyethylene bags (500 g), and irradiated to absorbed doses of 10.0 and 20.0 kGy using the ^{60}Co source at Sarayköy Nuclear Research Institute, Ankara. The dose rate was 6.0 kGy/hr. The absorbed dose was checked by Fricke dosimetry (Anonymous 1977). Unirradiated control samples and irradiated samples were stored for six months before laboratory analyses. Because the free radicals produced as a result of irradiation decay during the storage, there is no background signal from these radicals at the spectrometer conditions of the present study with the spin probe. Individual embryos of wheat kernels were excised with a stainless steel needle. Wholeness of embryos was checked by microscope. Moisture contents of the wheat samples were determined according to Approved Methods (AACC 1995). The nonviable embryos were prepared by keeping the wheat seeds for 17 hr at 110°C.

The spin probe 4-hydroxy-TEMPO (TANOL) was obtained from Aldrich. The aqueous solutions of this probe were prepared including a line-broadening material, potassium ferricyanide. A 10 mM aqueous solution of TANOL was prepared with 100 mM (solution I) and 200 mM (solution II) concentrations of potassium ferricyanide.

In all experiments, 1-mm i.d. capillaries were used. The bottom and top parts of capillaries were enclosed with parafilm M. EPR studies were performed using an X-band Varian E-9 spectrometer at 0.3 T.

The spectrometer conditions for spin probe studies were 100 kHz field modulation, 2 mW microwave power, room temperature, and modulation amplitude of 0.05 mT. Experiments with the spin probe were performed with single embryos of unirradiated control sample and with the embryos of the samples irradiated at levels of 10 and 20 kGy. A certain amount of the aqueous solution of the spin probe was taken into the capillary, then a spectrum of the solution without embryo was recorded. Next, the dry embryo was added to the capillary, and the time-dependent spectra were recorded. The signal intensity is followed as a function of time up to 150 min. In the rehydration curves, the differences between the first signal intensity (0') and the signal intensities recorded for the duration of the experiment were used. First signal intensity was the intensity recorded just after the insertion of the embryo. The experiments were repeated after two months.

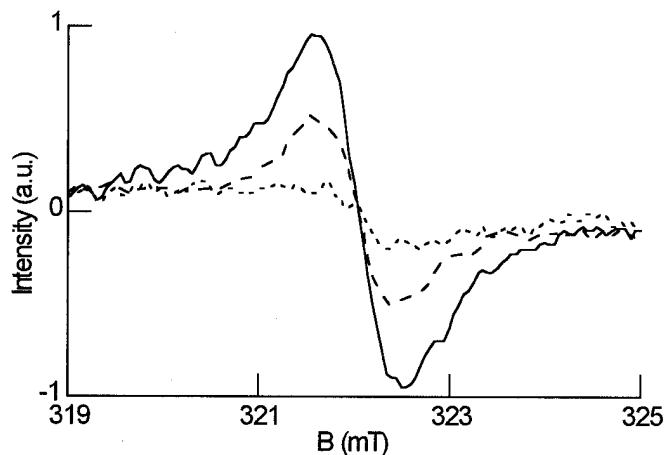


Fig. 2. Signal coming from the radicals produced as a result of irradiation background for unirradiated embryo (dotted line), 10 kGy (dashed line), 20 kGy (solid line).

RESULTS AND DISCUSSION

Before performing the experiments with the spin probes, the signals coming from the radicals produced as a result of the irradiation were tested. Tests were performed using single embryos of each sample. It was not possible to observe any signal at the spectrometer conditions given above. When modulation amplitude was changed to 0.2 mT and the receiver gain was increased ($\sim 10\times$), it was possible to record the spectra of single embryos (Fig. 2). The unirradiated sample gives a background signal and there is a variation in the signal intensity as a function of the dose. An increase in the number of embryos resulted in an increase in the signal intensity (not presented in the figure). After two months, the experiments were repeated and a decrease in the signal intensities of irradiated samples relative to background signal was observed. A similar decrease in the signal intensity of irradiated corn samples was also observed after 50 days of storage (Munoz et al 1994).

The spectra recorded with the use of solutions I and II for single embryos of the Bezostaya samples irradiated at 20 kGy level are given in Fig. 3A and B. Each spectrum observed is a superposition of the signals from the lipid and aqueous parts of the cells and the signal from extracellular region (broadened signal). Therefore, the high field line ($m_1 = -1$) signal is expected to differ from lipid and aqueous parts. A consideration of Fig. 3 indicates that it is possible to observe these signals when solution II is used. The superposing signals do not allow this observation for solution I.

The results obtained using solutions I and II for single embryos of unirradiated and irradiated Kunduru samples are given in Fig. 4A and B. This figure indicates that the signal intensity was higher for unirradiated embryo and decreased with the amount of the irradiation dose.

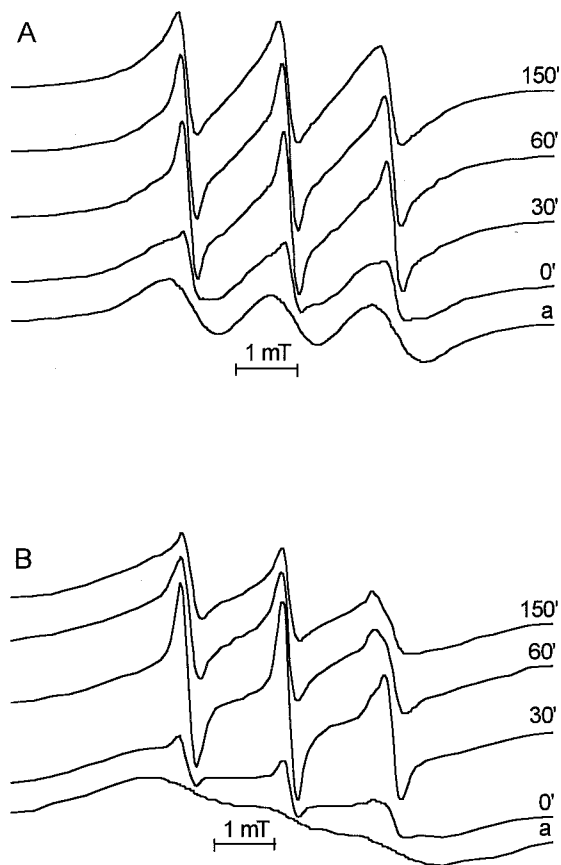


Fig. 3. Recorded spectra of single embryos at various times of rehydration of Bezostaya samples irradiated at 20 kGy level in solution I (A) and solution II. (B). a = Solutions without embryo.

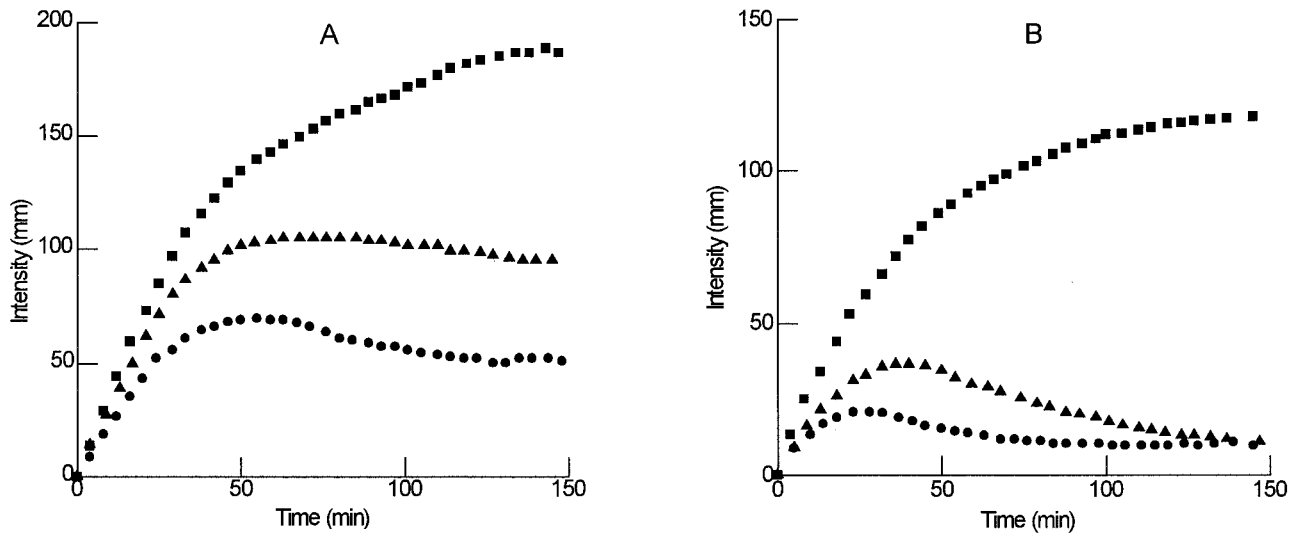


Fig. 4. Signal intensity time curves of single embryos (rehydration curves) of Kunduru samples in solution I (A) and solution II (B). ■ = Unirradiated, ▲ = irradiated at 10 kGy, ● = irradiated at 20 kGy.

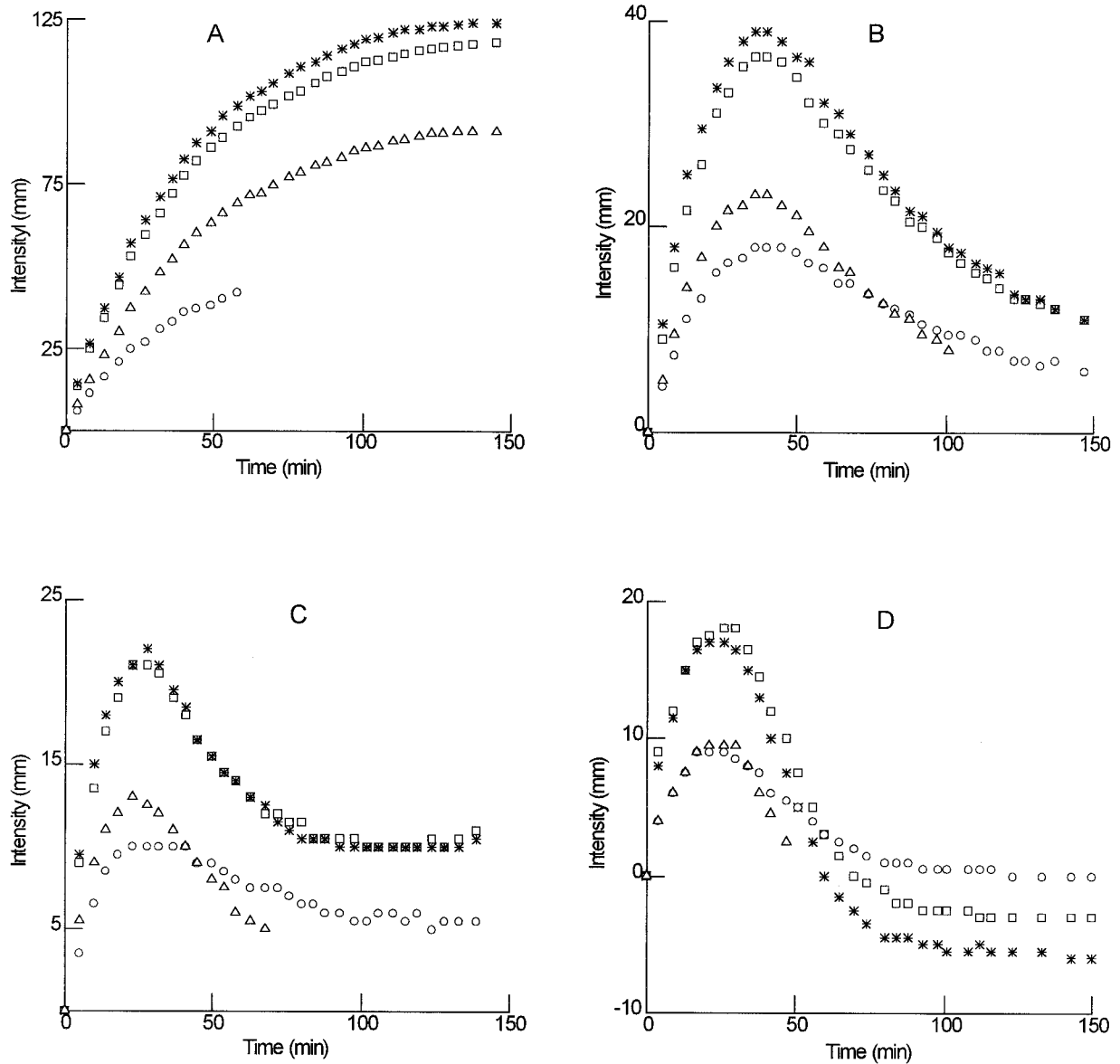


Fig. 5. Signal intensity time curves of Kunduru samples in solution II. A, unirradiated; B, irradiated at 10 kGy; C, irradiated at 20 kGy; D, nonviable wheat embryos.; $m_1 = +1$ (□), $m_1 = 0$ (*), and $m_1 = -1$ aqueous part (Δ), and lipid part (○), respectively.

Besides the change in the signal intensity, there was a change in the shape of rehydration curves. Similar results were obtained for both wheat cultivars. Although the signal intensity of the unirradiated embryo still had a tendency to increase at the end of 150 min, a decrease in the signal intensity was observed for the irradiated samples. This decrease started at ≈ 30 min and was observed at each hyperfine line on the spectrum. As a result of irradiation, the cell membrane probably loses its integrity and impermeability against broadening material. In recent studies (Golovina and Tikhonov 1994; Sünnetçioğlu et al, in press), the loss of membrane integrity was examined in aged seeds. Possibly the effect of irradiation is an artificial aging of the embryo and the degree of this aging depends on the irradiation dose.

In Fig. 5A–D, the intensity-time curves were given for samples that were unirradiated, and samples irradiated at 10 and 20 kGy, as well as nonviable Kündürü wheat samples in solution II. In all figures, the rehydration curves of the $m_1 = +1, 0, -1$ lines were given. Because the high field line was split into lipid and aqueous parts, the change of the signal intensity was followed for both parts. For the unirradiated sample, the rise of the signal intensity for the aqueous part was dominant and the signal from the lipid parts disappeared at ≈ 50 min. Contrary to this, for all other samples (Fig. 5B–D), the signal from the lipid parts could be followed until 150 min; the signal from the aqueous part was lost at ≈ 60 min. The loss of water signal can be explained by the presence of $K_3Fe(CN)_6$ inside the aqueous parts of the cells. As a result, the signal from the aqueous parts broadens and the signal intensity decreases. Because $K_3Fe(CN)_6$ does not penetrate into the lipid parts, the signal from these parts dominates and, after a while, the signal from the aqueous parts is lost. The broadening of the signal is especially striking for the nonviable sample (Fig. 5D); signal intensity decreases to a level below that of the beginning intensity. This is an indication of the complete loss of the membrane integrity.

Although utilization of EPR technique in the detection of irradiated grains appeared in previous literature (Onderdelinden and Strackee 1974, Raffi et al 1987, Hunter et al 1988, Troup et al 1989), to the best of our knowledge, application of EPR method with spin probe techniques for the detection of irradiated foods was used for the first time in this study. Despite the difficulty and complexity of the experiments with biological materials, the five repetitions of experiments gave rise similar results in terms of the shape and intensity. Therefore, this technique is promising in the detection of irradiated cereal samples. A combination of the studies with spectral simulation would shorten the experiment time and make the application possible for many samples.

CONCLUSION

Irradiation is a well-known technique in the preservation of foods. Increases in the international trade of irradiated foods have created a demand for detection methods that will provide regulatory agencies with the tools to ensure compliance with labeling regulations. EPR is a useful technique in the investigation of irradiated foods through the observation of the free radicals produced as a result of irradiation. The mean lifetimes of free radicals produced changes from days to months, depending on the hardness of the food. This stability is sufficient for the EPR investigation of some foods with short commercial storage times; however for cereals, the time of storage might be longer. In this case, the decay of free radicals makes the application of EPR technique impossible. There are no such time limitations for the EPR spin probe technique, however, and the samples can be investigated even after a longer period of storage. Therefore, this technique might be a detection method for investigating irradiated wheat and other cereals. Further experiments should be performed with a larger number of irradiation levels, including lower doses and also using different cereal grains before the EPR spin probe technique is recommended for practical applications.

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