Application of the Electron Paramagnetic Resonance Spin Probe Technique for Detection of Irradiated Wheat

M. Maral Sünnetçioğlu,[1,4] Dilek Dadayli,[2] Süeda Çelik,[3] and Hamit Köksel[3]

ABSTRACT

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The fast decay of free radicals makes application of traditional electron paramagnetic resonance (EPR) techniques impractical for detection of irradiated wheats when the storage time is longer than 30 days. In this study, therefore, the effects of irradiation on wheat seeds were investigated by using the EPR spin probe technique. The technique is based on the ability of the spin probes to transfer valuable information related to the changes in the structural characteristics of embryo cell membranes caused by irradiation. As a result of irradiation, the enviroment of the spin probe is modified and this modification can be followed from the recorded spectra. The doses studied were 1.0, 2.5, 10.0, and 20.0 kGy. An aqueous solution of 16-doxyl stearic acid (16-DSA) was used The embryos were detached using a steel needle and kept in 16-DSA solution for 2.5 hr and washed with distilled water. The spectra of the samples were recorded. The detection of irradiated wheats by this technique was possible at doses of ≥2.5 kGy. An important advantage of this approach is that it is applicable even after eight months of storage.

Irradiation using γ-rays has the potential to protect cereals from insect infestation and microbial contamination during storage. It has been reported that γ-irradiation doses in the range of 0.2–1.0 kGy are effective in controlling insect infestation in cereals (IAEA 1991). Increasing the dose to 5 kGy totally kills the spores of many fungi and bacteria surviving the lower doses (Murray 1990). Irradiation treatment of different foods is now legally accepted in many countries, although it is still prohibited in some. Development of convenient methods would be useful for determining whether a particular food had been irradiated and, if possible, at what dose (Bögl et al 1988).

Electron paramagnetic resonance (EPR) analysis has been shown to be a very useful tool in detecting the presence of stable free radicals in irradiated foods (Onderdelinden and Strackee 1974, Desrosiers and Simic 1988, Bögl 1989, Dodd et al 1989, Raffi and Angel 1989, Maloney et al 1992, Desrosiers 1996). The possibility of using EPR techniques for the detection of irradiated cereal samples within a certain period of storage has been investigated (Raffi et al 1987, Hunter et al 1988, Troup et al 1989, Köksel et al 1993, Munoz et al 1994). The concentrations of radicals produced by irradiation vary with food type. They were reported to be smallest for wheat and poppy seeds. The concentrations drop to below preirradiation levels in ≈30 days (Troup et al 1989, Pilbrow et al 1996). The decay of these radicals makes application of traditional EPR techniques impractical for the detection of irradiated wheats when the storage time is longer than 30 days.

When the number of free radicals is not high enough in a biological sample for the EPR application, it is possible to investigate the sample by using the EPR spin probe technique. This technique provides some specific data about molecular dynamic and structural organization of the system, as well as other parameters (Smirnov et al 1992). Spin probes or spin labels are stable free radicals containing unpaired electrons. They either move freely inside the environment from which they report information or stick in one part of the environment. In both cases, they are highly sensitive to their environments and they transfer valuable information about the changes in this environment without disturbing the system (McConnell and McFarland 1970, Berliner 1976). The application of the EPR spin probe technique to the investigation of plant seeds has a relatively short history. The first application was the investigation of the water content of wheat seeds (Kutscher et al 1986). Since then it has been applied as a test of viability of the seeds (Smirnov et al 1992, Golovina and Tikhonov 1994, Sünnetçioğlu et al 1997). The spin probe technique enables the investigation of the materials, which otherwise have insufficient numbers of free radicals to be detected by EPR spectroscopy. In a previous study (Dadayli et al 1997), wheat grain samples irradiated at 10 and 20 kGy doses were stored for six months. Afterward there were no background signals due to the free radicals from irradiation. Hence, utilization of the EPR technique for the detection of irradiated wheat was not possible when the samples were stored for six months after the radiation treatment. The EPR spin probe technique, however, was capable of distinguishing the same irradiated wheat sample from unirradiated ones. Therefore, the spin probe technique, using 4-hydroxy-TEMPO (TANOL), dedicated to the detection of irradiated seeds seems to be quite promising. The most important factor in the application of the spin probe technique to irradiated seeds is the time independence of the studies (Dadayli et al 1997).

TANOL is a commonly used hydrophilic spin probe because of its good water solubility. On the other hand compounds such as doxylstearic acid spin labels are hydrophobic and prefer the lipid regions. In these labels, the doxyl group is attached to different carbon atoms along the chain. Depending on the position of attachment of the doxyl group, the information received about the environment changes and the 16-doxyl-stearic acid (16-DSA) transfers information from the depth of membrane bilayers. Therefore it reflects the changes around its environment (i.e., inside the membranes of the embryo cells). The EPR signal of 1 m*M* 16-DSA in water consists of three well-resolved lines (m_I = 1, 0, –1) as a result of interaction of electron with the nitrogen nucleus. The changes in the recorded spectrum reveal information from the environment of the spin probe (Berliner 1976, Eaton and Eaton 1978).

Because of the success of the EPR spin probe technique in the detection of irradiated wheats in our earlier study (Dadayli et al 1997), in the present work we have attempted to examine the possibility of using 16-DSA for the detection of wheats irradiated at lower doses.

MATERIALS AND METHODS

The durum wheat sample (cv. Kunduru-1149) used in this study was obtained from the Field Crops Improvement Center, Ankara. The wheat samples were cleaned on a Carter Dockage Tester, placed in polyethylene bags (500 g) and irradiated with the doses of 1.0, 2.5, 10.0, and 20.0 kGy using γ-radiation from the ^{60}Co source at the Sarayköy Nuclear Research Institute, Ankara. The absorbed dose rate was 6.0 kGy/hr. The absorbed dose was checked by Fricke dosimetry (Chadwick et al 1977). The background signal coming from the radicals originated as a result of irradiation decays during storage (Dadayli et al 1997). Therefore, unirradiated control and irra-

[1] Dept. Physics Engineering, Hacettepe University, 06532, Beytepe, Ankara, Turkey.
[2] Dept. Physics, Zonguldak Karaelmas University, 67100, Zonguldak, Turkey.
[3] Dept Food Engineering, Hacettepe University, 06532, Beytepe, Ankara, Turkey.
[4] Corresponding author. E-mail: fmaral@eti.cc.hun.edu.tr Fax: 0 312 299 21 23.

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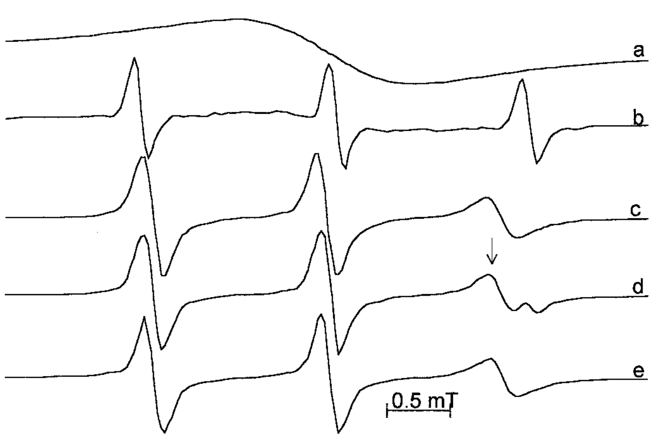

Fig. 1. Spectra of 16-doxyl stearic acid (16-DSA) powder (a), dissolved in water (1 m*M*) (b), and dissolved in soybean oil (1 m*M*) (c). Model experiments used solution of 16-DSA and pure soybean oil. Ratio of the 16-DSA solution to pure soybean oil at 2:1 (d) and 1:1 (e).

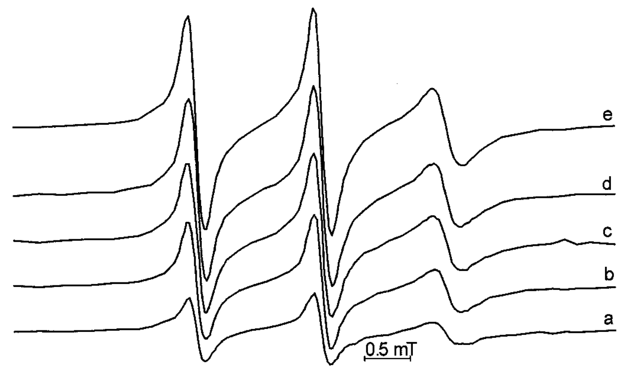

Fig. 2. Spectra of five embryos in the solution of 16-doxyl stearic acid (16-DSA) recorded using Method B just after the sample preparation: unirradiated (a) and irradiated at 1 kGy (b), 2.5 kGy (c), 10 kGy (d), 20 kGy (e).

diated samples were stored for four months before the EPR studies. Studies were performed using embryos of the wheat kernels. The embryos were detached using a steel needle without giving any harm to the wholeness of the embryos. In each case, they were controlled under the microscope to be certain that no damage had been done to the embryonic axes. Detachment of the embryos and the preparation of the samples for EPR measurements were performed as explained previously (Smirnov et al 1992, Sünnetçioğlu et al 1997).

The spin label 16-DSA was obtained from Aldrich Chemicals (Milwaukee, WI). A 1 m*M* aqueous solution of 16-DSA was prepared by magnetic stirring for 24 hr. Because of the low solubility of this spin label in water, some of it undergoes precipitation. Studies with the solution of 16-DSA were performed using two different preparation methods. In the first method (Method A), whole wheat kernels were soaked for 3 hr in distilled water. Five embryos were detached and kept in the 16-DSA solution for 0.5 hr. Then the embryos were washed in distilled water 15× without injuring them, using a tool made by covering one side of a small black hoop with black cheese-cloth. Finally, they were immediately transferred with a hair brush into Pyrex EPR tubes (3 mm i.d.) and the EPR spectra were recorded. In the second method (Method B), five embryos were kept for 2.5 hr in the 16-DSA solution and then washed in distilled water 15× as described above. In both Method A and Method B, the spectra of the samples were recorded just after sample preparation and also after drying the samples for 24 hr at room temperature to examine the effects of relaxation time on the samples.

The partition of the spin label in aqueous and lipid environments was tested by performing model experiments. For this purpose, the spin label was dissolved both in soybean oil and water separately at 1 m*M* concentrations, and the spectra were recorded. The hyperfine coupling constants were determined from the spectra. Afterward, certain amounts of pure soybean oil were added to an aqueous solution of 16-DSA in capillary tubes (1 mm i.d.) and the spectra were recorded for two different ratios (2:1 and 1:1) of the spin label solution to oil.

All of the analyses were repeated after four months and performed in triplicate. The spectra were recorded at room temperature by the use of a Varian E-9 X-band spectrometer with spectral conditions at modulation amplitude 0.05 mT and microwave power 2 mW.

RESULTS AND DISCUSSION

At the beginning of our studies with the 16-DSA, model experiments were performed to understand the partition of the label in hydrophobic and hydrophilic environments. The EPR spectra for powder and aqueous solutions of 16-DSA and the spectra recorded during the model experiments are presented in Fig. 1. The powder EPR spectrum of 16-DSA consists of a single broad resonance line (a), and the aqueous solution of 16-DSA gives a three-line spectrum (b). Since water is a polar solvent and soybean oil is a nonpolar solvent, hyperfine splitting constants and *g*-values are different. The localization of the unpaired electron on the nitrogen is higher for water and the hyperfine splitting constant is greater than that of soybean oil. This difference can be seen from the comparison of the high field lines ($m_I = -1$) in Fig. 1 (b and c). In model experiments, soybean oil and the solution of 16-DSA were used. First, various amounts of soybean oil were taken into a capillary and then the solution of spin label was added. The left part of the high field line corresponds to the signal from lipid parts (arrow in d), and the right part of it indicates the signal from aqueous parts in the system. The model experiments indicated that the signal from the lipid parts almost always dominates the signal from the aqueous parts (d and e).

An evaluation of the spectra of the samples prepared according to the Methods A and B, reveals an increase in the signal intensity and slight decrease in the line widths depending on the dose. Our recent study indicated the loss of the external membrane integrity due to irradiation (Dadayli et al 1997). Therefore, in the present study, the loss of membrane integrity due to irradiation might have caused the spin label to enter the cells easily, resulting in an increase in the signal intensity. The increase in the signal intensity depending on the dose was observed for both kinds of spectra recorded just after the sample preparation (Fig. 2) and after drying the samples for 24 hr (data not presented). The stability in the behavior of the signals after 24 hr might be an advantage for the practical application of the method and eliminate potential sources of error due to variation in the relaxation time of the sample. Our observations in this study concerning the practical aspects and the applicability led us to conclude that Method B, as a sample preparation method in which the embryos were kept in 16-DSA solution for 2.5 hr without presoaking in distilled water, is superior relative to Method A.

Another interesting result provided by the spectra of the samples prepared using Method B is the observation of the presence of a single-line signal superposed on the three-line spectrum of control and 1-kGy irradiated samples. This is illustrated in Fig. 3 as the difference between the normalised spectra of the control and of the 2.5-kGy irradiated samples. This single-line signal causes a baseline shift and the asymmetry of the $m_I = -1$ lines of both the control and 1-kGy irradiated samples. This asymmetry starts to disappear at the 2.5-kGy dose and the lines become more symmetrical with the increase in dose (Fig. 4). These changes were reproducible in the recorded spectra at all irradiation doses. This single-line signal might arise from the spin labels sticking to the membranes at the outer parts of the embryo. These labels appear to be in a solid-like environment and the sample gave a spectrum similar to the powder spectrum. The studies with 16-DSA suggest that the limit of detection

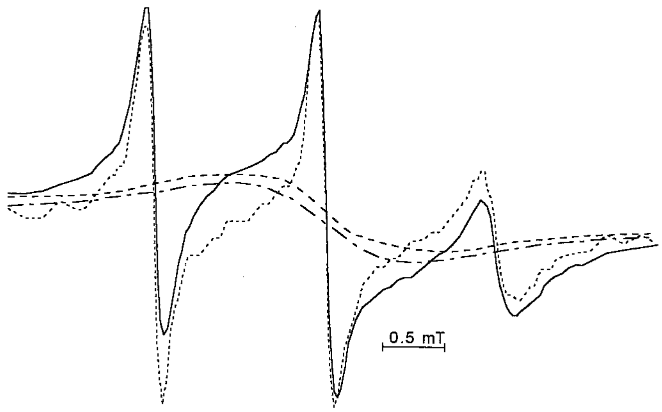

Fig. 3. Difference spectrum (dashed line) of control (solid line) and 2.5-kGy irradiated samples (dotted line) in the solution of 16-doxyl stearic acid (16-DSA) with Method B. Spectra are normalized according to m_I = –1 line. Powder spectrum of 16-DSA (dash and dotted line) is also included for comparison.

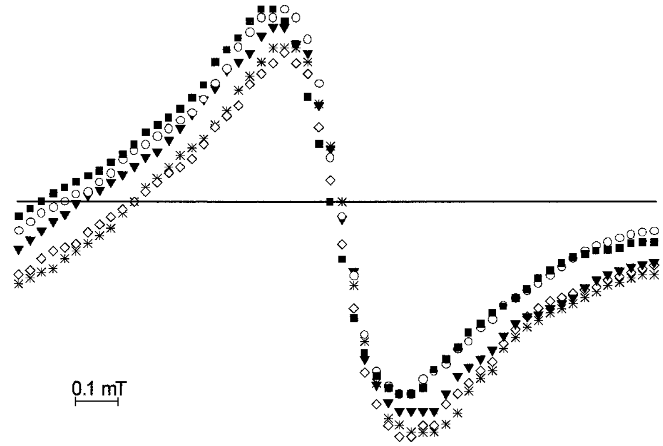

Fig. 4. Normalized spectra of m_I = –1 line of the samples in 16-doxyl stearic acid (16-DSA) solution recorded using Method B: unirradiated (✻), irradiated at 1 kGy (◇), 2.5 kGy (∇), 10 kGy (○), and 20 kGy (■).

of irradiation is as low as 2.5 kGy, and this method seems to have considerable potential for the detection of the irradiation in wheat.

The technique used in this study is different from our previous method (Dadayli et al 1997) in which TANOL was used with the line-broadening agent potassium ferricyanide to distinguish the signals of the spin probe from intra- and extracellular regions of embryo. However, in the present study, no line broadening agent was used because it was not needed. There is also an important advantage of using the spin label: There is no need to plot rehydration curves for 16-DSA, because evaluation of only one spectrum recorded after 150 min seems to be satisfactory to detect irradiated wheats, instead of recording spectra every 4 min for a period of 150 min. The behavior of signals were stable 24 hr after sample preparation. This might be another advantage that eliminates potential sources of error due to variation in the relaxation time of the sample. The method is based on the difference in baseline shift in the spectra and also disappearance of the asymmetry in highfield line at irradiation doses ≥2.5 kGy.

Applications of the EPR spin probes, especially for the study of detection of irradiation, are not common yet. To the best of our knowledge, in the present study, application of the EPR technique with the spin label (16-DSA) was used for the first time for the detection of irradiated grains.

CONCLUSIONS

EPR appears to be an excellent method for any foodstuff containing bones or cuticula, even in the absence of unirradiated controls (Dodd et al 1985, Bögl 1989). However, in cereals, the decay of the EPR signal is fast. It was reported that radical concentrations had decayed to ≈10% of initial postirradiation level in ≈30 days (Hunter et al 1988). Therefore the EPR technique can be used to detect irradiated cereals only within a certain period of storage. However, for the EPR spin probe technique, there is no such time limitation and the irradiated samples can be detected after longer periods of storage even in the absence of unirradiated controls. In a previous study, it was possible to detect irradiated wheats after eight months of storage by EPR spin probe technique using TANOL (Dadayli et al 1997). In the present study, another application of the EPR spin probe technique was tested using a spin label. It can be concluded that Method B, in which the embryos were kept in 16-DSA solution for 2.5 hr without presoaking is superior as a sample preparation method relative to Method A in terms of applicability. The limit of detection of irradiated wheats by this technique was 2.5 kGy. The detection was possible even after a storage period of eight months. However, more work is necessary to improve the method and the sensitivity before EPR spin probe technique can be recommended for industrial application.

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