Estimation of the Absorbed Dose in Radiation-Processed Food. 4. EPR Measurements on Eggshell

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Fresh whole eggs were treated with ionizing radiation for Salmonellae control testing. The eggshell was then removed and examined by electron paramagnetic resonance (EPR) spectroscopy to determine if EPR could be used to (1) distinguish irradiated from unirradiated eggs and (2) assess the absorbed dose. No EPR signals were detected in unirradiated eggs, while strong signals were measurable for more than 200 days after irradiation. Although a number of EPR signals were measured, the most intense resonance ($g = 2.0019$) was used for dosimetry throughout the study. This signal was observed to increase linearly with dose (up to ~6 kGy), which decayed ~20% within the first 5 days after irradiation and remained relatively constant thereafter. The standard added-dose method was used to assess, retrospectively, the dose to eggs processed at 0.2, 0.7, and 1.4 kGy. Relatively good results were obtained when measurement was made on the day the shell was reirradiated; with this procedure estimates were better for shell processed at the lower doses.

INTRODUCTION

Electron paramagnetic resonance (EPR) has been advanced as a rapid and accurate method to distinguish irradiated from unirradiated foods. The EPR method is based on the measurement of long-lived chemical species with unpaired electrons (referred to here as paramagnetic centers) produced in the hard matrices of food by the absorption of ionizing radiation. The most successful applications of the EPR method have used bone in meat (Desrosiers *et al.*, 1990) or the shell of molluscs and crustacea (Desrosiers, 1989; Raffi and Agnel, 1990; Morehouse and Desrosiers, 1993; Stewart *et al.*, 1993) as radiation detectors. Limited successes have been achieved for the use of seeds in fruit (Raffi and Agnel, 1989; Desrosiers and McLaughlin, 1989) and spices (Yang *et al.*, 1987; Uchiyama *et al.*, 1990) treated with ionizing radiation. Dodd *et al.* (1988) further demonstrated that the EPR method could be used quantitatively to estimate the absorbed dose in radiation-processed foods containing bone; moreover, recent refinements have led to improvements (Desrosiers, 1990, 1991a,b; Desrosiers *et al.*, 1991; Desrosiers and Le, 1993).

The primary reason for treating poultry with ionizing radiation is to control Salmonellae and other pathogens. Fresh whole eggs in intact shells are another source of Salmonellae contamination, with a number of outbreaks of salmonellosis resulting from the consumption of raw or undercooked eggs.

To assess whether radiation treatment would reduce the microbial hazard without significantly affecting the nutritional and organoleptic quality of fresh whole eggs, a joint study was undertaken at the University of Rhode Island (URI) and the University of Massachusetts at Lowell (UMass–Lowell). It was recognized by these researchers that eggshell may be a suitable material for examination by EPR to identify irradiated eggs and their absorbed dose.

A previous study on eggshell irradiated from 1 to 10 kGy demonstrated that EPR could be used to distinguish irradiated eggs (Kiyak, 1990). It was shown that a long-lived radiation-induced paramagnetic center in eggshells was produced proportionally with dose and persisted even after the eggshell was heated to 100 °C for 1 h.

As an adjunct study, eggshell from eggs used in the URI and UMass–Lowell study were sent to NIST for EPR measurements. We describe here a detailed analysis on the dose dependence and long-term stability of EPR signals in irradiated eggshell, as well as the use of EPR to estimate retrospectively the processing dose.

EXPERIMENTAL PROCEDURES

Chicken eggs (1 day old) in intact shells, all laid by the same strain of hens, were obtained from an egg farm in Rhode Island. The eggs were packaged in egg cartons within insulated containers (chilled and buried in cracked ice) and transported by automobile (approximately 2 h) to the University of Massachusetts at Lowell, MA, where they were treated with ^{60}Co gamma radiation. Three different egg cartons, each containing four eggs, were positioned at three different distances (20, 36, 71 cm) in the Gamma Cave Facility and irradiated for 20 min. The eggs were rotated 180° halfway through the irradiation. The absorbed dose, determined by Optichromic dosimeters, at each distance was 0.2, 0.7, and 1.4 kGy. While in the irradiation chamber, the eggs were kept chilled by commercially available frozen chilled packs. After the irradiation, the eggs were transported (chilled and buried in cracked ice) to the University of Rhode Island, where they were stored at approximately 4 °C for subsequent analysis.

After removal, shells from eggs used for organoleptic analysis were set aside in a desiccator until shipment to NIST for EPR analysis. There were four lots of shells, one from each absorbed dose, and one representing a control (unirradiated).

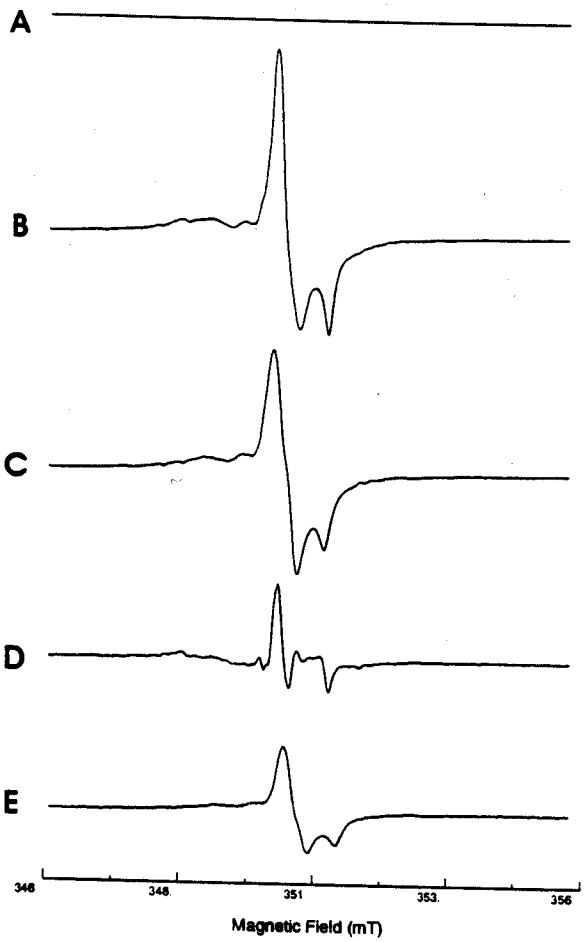

Figure 1. First derivative EPR spectrum of eggshell: (A) unirradiated; (B) 1.0 kGy, immediately after irradiation; (C) 1.0 kGy, 23 days after irradiation; (D) spectrum B minus spectrum C; (E) irradiated to 1.0 kGy and stored at 4 °C for 349 days. Spectrum E is 4 times the scale of spectra A–D.

Unirradiated eggshell was both mortar ground (approximately 1–2-mm diameter) or mechanically milled and passed through a 1-mm sieve to examine the effect, if any, that milling the shell would have on the EPR spectrum. Each batch of irradiated shell was individually milled and sieved. Only milled shell was used for the EPR dose assessment.

For each batch of radiation-processed shell approximately 2 g of shell was placed into seven separate capped vials. Each of six of the vials was irradiated to one of the following nominal absorbed doses: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 kGy. For these and subsequent irradiations, a Gammacell 220 ^{60}Co source ($D_{H_2O} \simeq$ 7 kGy/h) was used. Approximately 0.5 g of shell was transferred from each vial to a quartz EPR tube and packed to a height sufficient to fill the height of the microwave resonator (approximately 3–4 cm). The EPR measurements were made using an ESP300E X-band EPR spectrometer. The spectrometer settings were as follows: modulation amplitude, 0.10 mT (1.0 G); microwave frequency, 9.8 GHz (modulated at 100 kHz); microwave power, 10 mW. Reported EPR signal intensities are derived from the peak-to-peak distance of the EPR resonance in the first-derivative spectrum and normalized by the diameter of the EPR tube.

RESULTS AND DISCUSSION

The EPR spectrum of mechanically milled eggshell (Figure 1A) was indistinguishable from that of the mortar ground shell; in fact, there were no detectable EPR signals for either sample. A complex EPR spectrum was observed for gamma-irradiated (1.0 kGy) eggshell (Figure 1B). Figure 1C is an EPR spectrum of the ground irradiated eggshell recorded 23 days after irradiation. The EPR spectrum in Figure 1D is a difference spectrum obtained by subtracting the spectrum in Figure 1C from that in Figure 1B. This spectrum (D) was dominated by a major resonance at $g = 2.0019$ ($\Delta H_{p-p} = 0.28$ mT) and a minor resonance upfield at $g = 1.9969$. It is important to note that the $g = 2.0019$ resonance in Figure 1B has an apparent line width of 0.42 mT (ΔH_{p-p}) due to an unresolvable weak signal superimposed on the high-field side of this resonance. A weak resonance at $g = 2.0034$ ($\Delta H_{p-p} = 0.06$ mT) was detectable at low microwave power (<1 mW) (not shown). The intensity of this resonance reached a maximum at doses of ~1 kGy and was not studied further. These spectral features are comparable to those previously observed in irradiated carbonates and apatites and are consistent with a shell composition dominated by $CaCO_3$. The $g = 2.0019$ and 1.9969 resonances are similar to those assigned to carbonate radicals in apatites (Rossi *et al.*, 1989) and identical to those observed in irradiated eggshell (Kiyak, 1990). The weak resonance at $g = 2.0034$ was not reported by Kiyak and is tentatively assigned here as the CO_3^{3-} radical ($g = 2.0034$ and 2.0017) reported in irradiated carbonates by Serway and Marshall (1967). The $g = 2.0017$ component would be obscured by the intense $g = 2.0019$ resonance. The resonance that contributes to the linewidth broadening in Figure 1B is more apparent in Figure 1C. From this spectrum we calculate a $g = 2.0007$, which has been previously assigned to an isotropic CO_2^- radical (Miki and Kai, 1990).

The peak-to-peak intensity of the $g = 2.0019$ resonance for eggshell irradiated to three doses (0.3, 1.5, 5.0 kGy) was measured over the course of 23 days (stored at ambient temperature); these data are shown in Figure 2. It was found that the rate of signal decay was only slightly dependent on dose. However, when the same shell was reirradiated with an identical dose 8 days later, the EPR signal decayed at a slower rate overall, but this rate was more pronounced with increasing dose. The overlapping resonances that contribute to the intensity of the measured EPR signal intensity likely influence the apparent signal decay. The dose dependence and decay of the interfering signals are not known, and attempts to spectroscopically eliminate or minimize the underlying signal(s) were unsuccessful.

Fresh whole eggs purchased from a local Maryland market were irradiated to 1 kGy at ambient laboratory temperature and stored at 4 °C. On days 23, 48, 205, and 349 the eggs were broken open and the embryo and inner membrane discarded. The shell was air-dried and milled. Radiation-induced EPR signals were detected on each of these days; the EPR spectrum for the egg examined on day 349 is shown in Figure 1E. The strong signals measured over this time period indicate that the longevity of the EPR test far exceeds the shelf life of fresh eggs.

Figure 3 shows the EPR spectra for the three batches of irradiated eggshells received from URI. For each of the three batches of irradiated eggs, six aliquots were irradiated to added doses in 0.5-kGy increments (see Experimental Procedures). The EPR spectrum for each sample was measured on the day of irradiation (day 1) and on days 5 and 8. The peak-to-peak intensity of the $g = 2.0019$ EPR resonance was measured and plotted as a function of added dose (Figures 4–6). A least-squares fit was made to each data set, and the function was extrapolated to the negative dose axis (abscissa). The x intercept is an estimate of the processing or initial dose. The extrapolated values are summarized in Table I.

For each batch of irradiated eggshell a decay of the EPR signal for each added dose led to dose estimates which varied with time. The data for day 5 were removed for

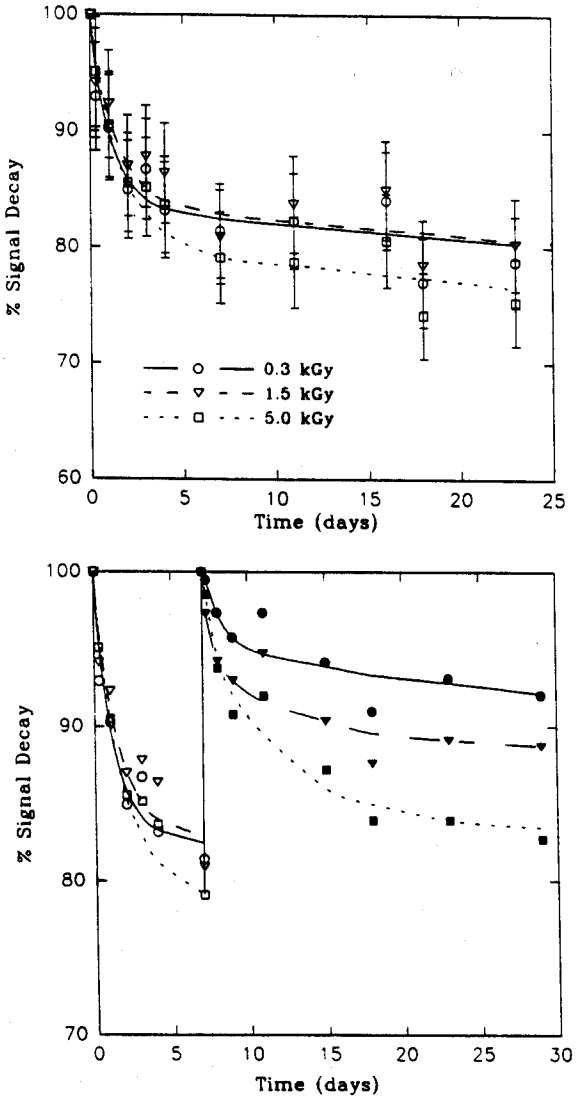

Figure 2. Time-dependent change in radiation-induced EPR signal intensity for eggshell expressed as percent change in the signal measured immediately after irradiation: (A, top) 0.3 kGy (○), 1.5 kGy (▽), 5.0 kGy (□); (B, bottom) days 0–8, 0.3 kGy (○), 1.5 kGy (▽), 5.0 kGy (□); on day 8 each was reirradiated with the same dose, 0.6 kGy (●), 3.0 kGy (▼), 10.0 kGy (■).

clarity in Figures 5 and 6; the dose estimates for these data are in Table I. A small variation in the estimated dose was noted for the 0.2-kGy eggshell. However, the agreement between the processing dose and the estimated dose became progressively worse as the time of measurement increased.

To determine which data yield the most reliable estimate, unirradiated eggshell powder was given a 1.0-kGy dose. Eight days later, the powder was divided into seven portions, six of which were reirradiated to doses of 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 kGy. The EPR spectra were measured at each dose, and the initial dose (known to be 1.0 kGy) was determined by back-extrapolation of the least-squares fit to the data. Eight days after reirradiation, the EPR spectra were remeasured for all seven samples and the initial dose was recalculated by back-extrapolation. The data are shown in Figure 7. The best estimate was obtained on the first day (1.0 ± 0.1 kGy), an overestimate (1.3 ± 0.2 kGy) was obtained for spectra measured 8 days after the reirradiation. The difference in the estimates was reduced by ~50% (estimated dose = 1.17 kGy) when the EPR intensity data at 8 days were adjusted for the time-dependent decay using data in Figure 2B (this

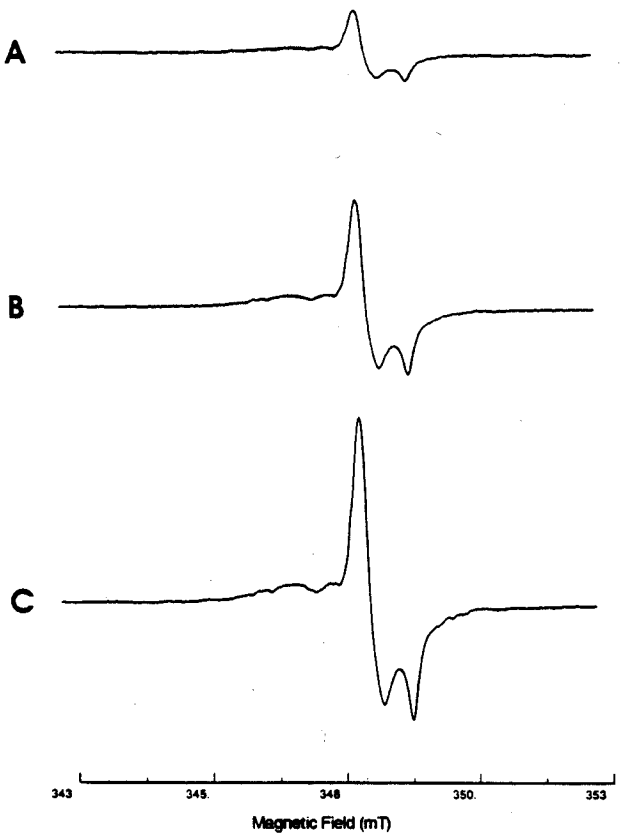

Figure 3. Eggshell EPR spectra for eggs processed at (A) 0.2 kGy, (B) 0.7 kGy, (C) 1.4 kGy.

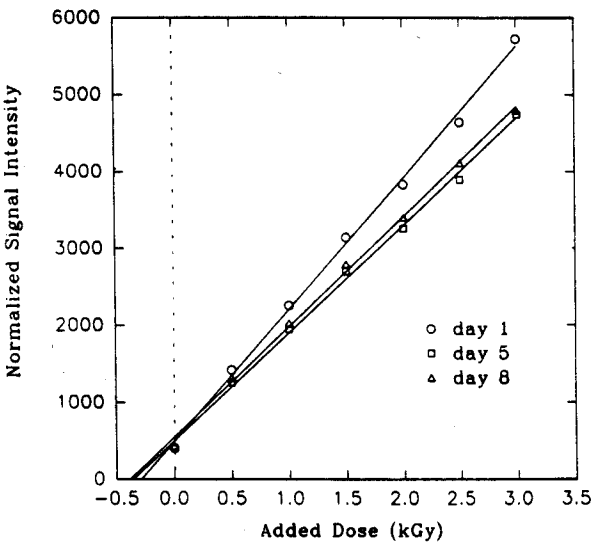

Figure 4. EPR signal intensity for 0.2 kGy processed eggs as a function of added dose, measured on the day of irradiation (○) and 5 (□) and 8 days (△) after irradiation. The lines are least-squares fits to the data.

adjustment is not shown). Therefore, if the EPR spectrum of eggshell is not measured on the day it is reirradiated, correction factors accounting for the short-term decay must be determined and applied to the EPR signal intensities used to obtain the dose estimate.

The EPR signals produced by ionizing radiation are easily detectable even at the lowest processing dose, 0.2 kGy. In fact, signal-to-noise measurements at this dose with a higher microwave power (200 mW) suggest a limit of detection of at least 0.5 Gy. The EPR signal longevity is likely to far exceed the storage life of whole eggs. However, the complexity of the EPR spectrum along with

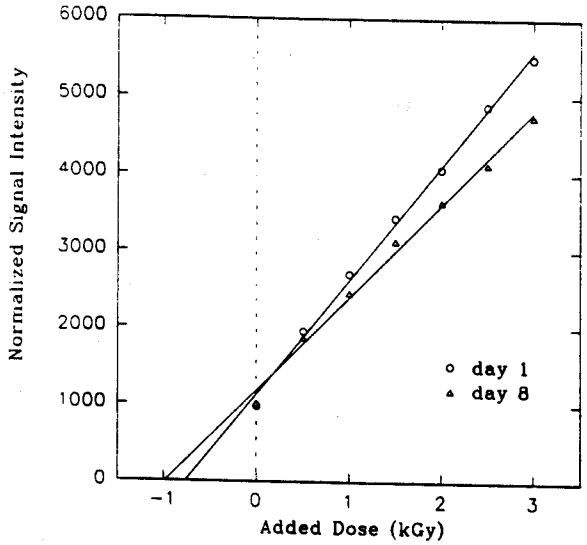

Figure 5. EPR signal intensity for 0.7 kGy processed eggs as a function of added dose, measured on the day of irradiation (○) and 8 days (△) after irradiation. The lines are least-squares fits to the data.

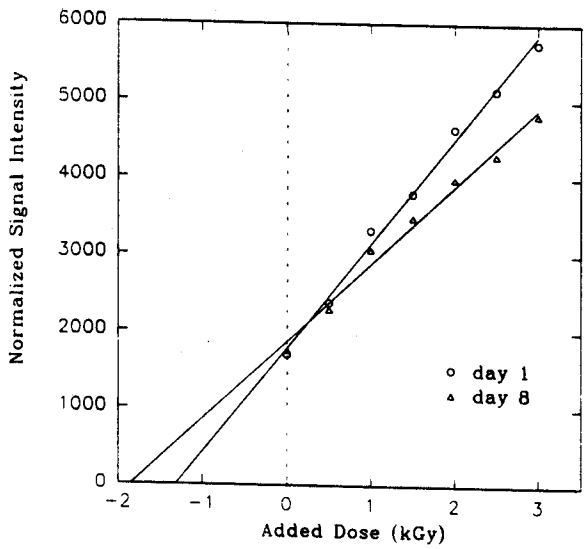

Figure 6. EPR signal intensity for 1.4 kGy processed eggs as a function of added dose, measured on the day of irradiation (○) and 8 days (△) after irradiation. The lines are least-squares fits to the data.

Table I. Irradiated Eggshell: Comparison of the Dose Estimated by the EPR Method on the Day of Reirradiation and Remeasured on Days 5 and 8

processing dose,[a] kGy	extrapolated dose,[b] kGy		
	day 1	day 5	day 8
0.2	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
0.7	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
1.4	1.3 ± 0.2	1.6 ± 0.2	1.9 ± 0.2
1.0[c]	1.0 ± 0.1		1.3 ± 0.2

[a] Estimated overall uncertainty is ±2.2% at a 95% confidence level. [b] Error is twice the standard deviation of the extrapolated x intercept and was calculated according to the "bootstrap" method (Efron and Tibshirani, 1986). [c] Eggshell powder irradiated at NIST.

the short-term decay characteristics of the signals make quantitation difficult. Since the eggshell used in the EPR measurements was derived from large numbers of eggs ground together to form one sample, contributions from sample-dependent variables may also influence the estimate. Another factor may be the influence of moisture content at the time of irradiation, i.e., whole egg vs shell powder. The estimates from the eggshell powder irradiated at NIST may indicate that quantitative measurements are most accurate when completed in 1 day, and if not, correction factors must be applied to account for dose-dependent decay. The failure of the quantitative test at higher doses correlates with dose-dependent signal-decay data. This observation, along with the fact that the measured signal is actually a composite of two or more EPR resonances, is likely to be the most influential factor for the decrease in measurement confidence at high doses.

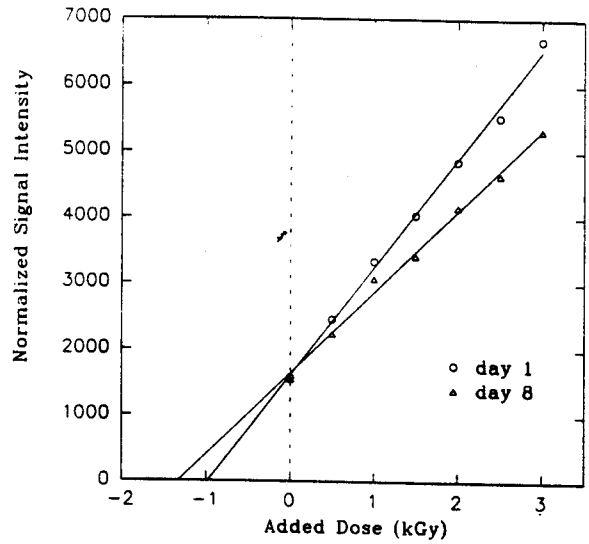

Figure 7. EPR signal intensity for 1.0 kGy irradiated eggshell as a function of added dose, measured on the day of irradiation (○) and 8 days (△) after irradiation. The lines are least-squares fits to the data.

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