Registry No. $NaHCO_3$, 144-55-8; aluminum, 7429-90-5; acetic acid, 64-19-7.

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Postirradiation Dosimetry of Meat by Electron Spin Resonance Spectroscopy of Bones

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Electron spin resonance (ESR) spectroscopy was used to measure the production of free radicals induced by ^{60}Co γ-rays in chicken bones. It was found that the radiation-induced ESR signal in bone could easily be distinguished from the endogenous ESR signal. Long-term (4 months) stability studies at 20 °C showed no decay of the radiation-induced ESR signal. A linear relationship was observed between the radiation-induced ESR signal intensity (peak-to-peak amplitude) and the absorbed dose (1–5 kGy). It was concluded that ESR measurements of bones can be used to determine whether the bone-containing meat has been irradiated and also at approximately what dose. The measurements indicate the feasibility of postirradiation dosimetry (PID) of meats when bones are present.

The treatment of natural and processed food products by ionizing radiation has been under consideration as a method of food preservation for the past 30 years (Goresline, 1983). The United States Food & Drug Administration has approved irradiation of foods up to an absorbed dose of 1 kGy (100 krads) (*Federal Register*, 1986), whereas the World Health Organization has set considerably higher limits at 10 kGy (1 Mrad).

A controversial aspect of food irradiation processing is the possible creation of radiolytic products unique to irradiated foods whose safety has not been assessed. Until these issues are resolved, an important aspect of food technology from the standpoint of safety and quality control is the development of convenient and reliable methods for determining whether a particular food item has been irradiated and at what dose. The measurement of dose after the fact by quantitative analysis of the specific radiation-induced changes in the specimen in question will be referred to here as postirradiation dosimetry (PID).

An important aspect of PID is the development of a highly specific marker that would serve as an internal dosimeter inherent to that particular food item. An ideal choice for this purpose would be a unique radiolytic product of the food item that changes linearly with the application of ionizing radiation over a required dose range and does not fade away during the lifetime of the food item. Initial studies into such techniques have already shown feasibility (Karam and Simic, 1986). The technique is based on generation of radiation-induced *o*-tyrosine (*o*-Tyr) from phenylalanine in meats such as chicken. The amount of *o*-Tyr, as determined by gas chromatography/mass spectrometry, in irradiated meat increases linearly with dose (0.5–5 kGy) (Karam and Simic, 1986) Though promising, the technique, or for that matter any one particular technique, cannot be expected to be applicable to all types of foods, e.g. all meats, fruits, spices, etc. Therefore, it is necessary to develop an array of techniques that can be used to maintain quality control

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for a wide variety of food items. In addition, more than one radiation detection method for a particular type of food item may be desirable in order to have a backup or alternative check for confirmation of detected radiation exposure.

An alternative technique is electron spin resonance (ESR) spectroscopy (Gordy et al., 1955; Swartz, 1965; Onderdelinden and Strackee, 1974; Dodd et al., 1985). Radiation-induced free radicals produced in hard matrices of foods (bones, shell) become trapped and may be monitored by ESR, since the ESR signals may persist for long periods of time (Ikeya and Miki, 1980). The yield of radiation-induced free radicals can be quantified by ESR and therefore may be correlated with the dose absorbed by the bone. In fact, an accurate dosimetric technique based on ESR analysis of radiation-induced free radicals in crystalline amino acids bound in paraffin matrices has been established (Regulla and Deffner, 1982). Furthermore, postirradiation dosimetry by ESR of tooth enamel (Ikeya et al., 1984) and inanimate objects (Sastuen et al., 1983) has been used to accurately determine the dose absorbed by human subjects subjected to radiation exposures. The technique has been proposed as a means of emergency dosimetry when conventional radiation protection dosimeters have not been used (Nakajima, 1982). In this study, we have further applied ESR spectroscopy to facilitate PID measurements of radiation-processed meats.

EXPERIMENTAL SECTION

Fresh, never frozen, chicken parts were irradiated by a ^{60}Co source at two absorbed dose rates: 0.0683 or 0.180 kGy/min. The meat samples were held in Pyrex beakers for the irradiation step. Bones from various chicken parts were irradiated both with and without the attached meat. The excised bone was vacuum-desiccated at ambient temperature overnight, cut, and weighed. Attempts were made to obtain similar sample weights (typically 150 ± 30 mg). The bond samples were stored in a desiccator at 20 °C (oxygen was not excluded). ESR spectra were recorded with a Varian E-109 X-band spectrometer at 20 °C. For these studies the microwave power used was 10 mW. The position of the radiation-induced ESR signal was compared with that of the standard 2,2-diphenyl-1-picrylhydrazyl (Aldrich) for accurate measurement of the spectroscopic splitting factor, g. The error limits for the measured g values were estimated to be ±0.03% (σ). ^{60}Co radiation source was calibrated by Fricke dosimetry.

RESULTS AND DISCUSSION

The observed radiation-induced ESR signal in the bone (Figure 1A,B) is due to an asymmetric singlet with $g_\perp$ = 2.0022 and $g_\parallel$ = 1.9980. These values are in good agreement with those previously reported for irradiated bone ($g_\perp$ = 2.0023) (Houben, 1971). Previous investigations (Gordy et al., 1955; Swartz, 1965; Houben, 1971; Stachowicz et al., 1970; Fisher et al., 1971) designed to deduce the precise nature of the free-radical signals in bone have proved inconclusive. However, the radiation-induced ESR signals produced in bone (in air, at ambient temperature) are currently regarded (Houben, 1971; Stachowicz et al., 1970; Fisher et al., 1971) as arising from lattice defects in the inorganic matrix of bone created by interaction with ionizing radiation.

Our investigations have demonstrated that the radiation-induced ESR signal in the bone of chicken can easily be distinguished from the endogenous signal. A comparison of parts A or B with part C of Figure 1 clearly shows that even at the lowest dose applied (1 kGy) the radiation-induced signal is easily discernible. From these data we are able to estimate the lowest detection limit (under these experimental conditions) for irradiated chicken bone to be about 0.1 kGy. This estimate is conserative considering that much lower doses have been measured in bovine bone (0.5 Gy) (Caracelli et al., 1986). As observed in Figure 1C, a small broad singlet (g = 2.0043; line width 8 G) was present in the nonirradiated control sample. This signal, though partially obscured by the more intense radiation-induced signal, is also observed to the low-field side of the ESR spectra in Figure 1A,B. However, no discernible change in the intensity of the endogenous signal is observed even at the highest dose absorbed (5 kGy).

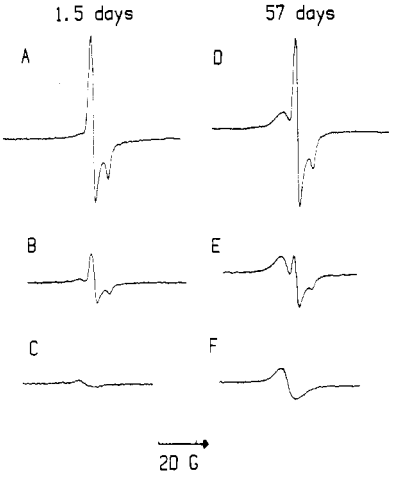

Figure 1. ESR spectra of irradiated (A = 4 kGy, B = 1 kGy) and nonirradiated (C) chicken thigh bone pieces recorded at 20 °C. Spectra were recorded at the same instrument gain setting, but the intensities are not normalized with respect to the individual sample weights. The spectra for each dose are of the same bone sample measured at two different time intervals (A–C = 1.5 days, D–F = 57 days); t = 0 is defined as the time at which the bone was irradiated. The bone samples were stored at 20 °C. Horizontal scale shown is in gauss (G); the arrow is in the direction of increasing field.

Comparison of the radiation-induced ESR signal in chicken (thigh) bone measured at 36 h and 57 days after irradiation (Figure 1, parts A, B vs. D, E) reveal that they are essentially identical. Continued measurements have shown this to be true even up to 4 months. No definitive loss of the radiation-induced ESR signal amplitude was observed during this period despite the fact that the storage temperature (20 °C) was higher than typical refrigeration temperature. Curiously, the intensity of the ESR signal for the nonirradiated bone was observed to increase with length of storage time at 20 °C. The endogeneous signal has been observed previously, and its origin is presumed to arise from an as yet unidentified organic component of the bone (Houben, 1971; Caracelli et al., 1986).

The intensity of the radiation-induced ESR signal ($g_\perp$ = 2.0022) was measured, and the data are represented as signal intensity to sample weight ratios and corrected for any differences in instrument gain. The change in ESR signal intensity (peak-to-peak amplitude) was then plotted as a function of absorbed dose (Figure 2). A linear relationship is observed between the increase in the radiation-induced ESR signal in the dose range measured. The error bars represent the standard deviation of the mean ESR signal intensity for bone samples measured at each dose level (at least three samples). The mean value was then plotted. Small variations in the intensity for some of the individual recorded ESR signals are observed over the course of the time-dependent measurements (60 days); however, the linearity and slope of the dose–response plot were preserved throughout the measurement period. The

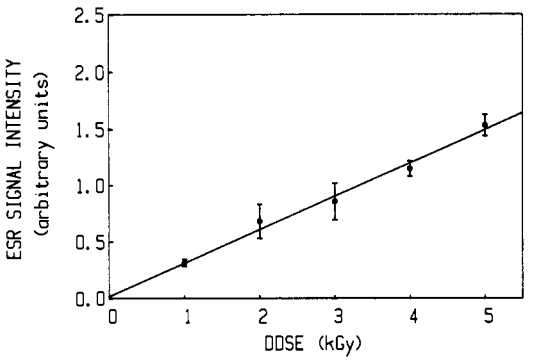

Figure 2. ESR signal amplitude (peak-to-peak) of the radiation-induced signal plotted as a function of the absorbed dose in the surrounding chicken thigh muscle. The error bars represent the standard deviation of the mean ESR signal intensity for bone samples measured at each dose level. Data points represent the calculated mean value for the data at each dose measurement.

intensity of the radiation-induced ESR signal and thus the slope of the line may be a function of the crystallinity and/or degree of mineralization of the bone (Ostrowski et al., 1972). Recently acquired data for γ-irradiated bones in meat (Desrosiers and Simic, unpublished results) may support this hypothesis.

Chicken bone irradiated with the surrounding meat attached produced ESR signals equal in magnitude (within our experimental limits) to those of the excised bone. This is because attenuation of the γ-rays by the meat is negligible and interaction of bone and meat free radicals does not play a role. With regard to the relative absorbed dose in the bone and in the meat, based on the relative values for the mass energy-absorption coefficients (Hubbel, 1982) of the two materials (calculated by methods described previously (Miller and McLaughlin, 1983) using the integral of the approximate degraded ^{60}Co source energy spectrum (Woolf and Burke, 1984)), the absorbed dose in bone is 3.4% less than that of the surrounding meat. The dose values in Figure 2 are those of the absorbed dose in the meat.

Radiation-induced ESR signals were observed in other bones (breast, rib) of the chicken, and identical spectra were obtained. Cartilage irradiated in its natural, wet state and then dried gave a signal (singlet, $g = 2.0054$, line width 10 G) that was different from that observed in bone but was indistinguishable from the ESR spectrum of the nonirradiated cartilage. Consequently, chicken cartilage cannot be used for PID by this method.

The mechanism(s) for generation of signal/species (e.g., the effect of irradiation parameters), radiation specificity of these signals, and the parameters affecting the lifetime of these signals are not fully understood. Therefore, studies to determine the effects of irradiation and storage (e.g., humidity, temperature, etc.) parameters on the radiation-induced ESR signal intensity must be performed to further refine this technique. These studies are necessary to develop what appears to be an excellent technique for PID analysis of irradiated meats that contain at least a small (few milligrams) piece of bone.

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