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ESR Signal in Different Cuts of Irradiated Chicken, Pork and Beef

Hye-Seon Nam and Jae-Seung Yang
Korea Atomic Energy Research Institute,
150 Dukjin-dong, Yusong-gu, Taejeon 305-353, Korea
jsyang2@nanum.kaeri.re.kr

Sun-Yung Ly
Chungnam National University,
220 Kung-dong, Yusong-gu, Taejeon 305-764, Korea

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Abstract

Electron spin resonance (ESR) spectroscopy was used to detect irradiated meat containing bones (chicken, pork and beef), to investigate the effect of irradiation dose on the ESR signal intensity and to identify the stability of radicals under 9 weeks of storage. Chicken, pork and beef were irradiated with doses 0, 1, 3, 5 and 7 kGy at room temperature using a Co-60 irradiator. Bones were pieced and dried, which were placed in a quartz tube within an Electron paramagnetic resonance (EPR) spectrometer resonator cavity. The irradiated bone presented an asymmetric absorption in shape, different from that of a non-irradiated one. The signal intensity of smaller animals are lower than larger species. Variation was observed between samples of the same species depending on the calcification status of the bone. Moreover different irradiation doses produced different signal areas that make possible to estimate the absorbed dose of treated meat. The ESR signal stability after irradiation was stable in even after a 9 week storage at room temperature.

Key Words : ESR spectroscopy, signal intensity, signal stability, chicken, pork, beef

1. Introduction

The use of ionizing radiation for the preservation of food is not a new technology. Since the 1950's, many countries have been involved in the evaluation of technology for the preservation of a wide range of foods and accordingly, the process is used commercially in a number of countries. The process kills spoilage microorganisms and so the shelf-life of these foods can be extended. The

ripening of fruits can be delayed by irradiation and the technology provides an alternative to the chemicals, which have been or are still being used to decontaminate spices and herbs, disinfest cereal and tropical fruit, and inhibit sprouting in tubers such as potatoes(1). Moreover, in combination with good hygienic practices, the process is effective in enhancing food safety by reducing the numbers of pathogenic microorganism including *E. coli* O157:H7, *Listeria, Salmonella and Campylobacter*, which are often implicated in food poisoning outbreaks involving poultry, meat, fish and shellfish(2).

However, in most cases there are regulations about which kinds of products are eligible for treatment and what is the maximum dose. In some countries, such food treated by ionizing radiation is prohibited. Therefore clear, unambiguous labeling and enforcement of the labeling claims through an existence of a reliable range of detection tests would assist consumer acceptance of the process.

To date a number of tests have been developed as reliable methods for the identification of irradiated meat by thermoluminescence, detection of 2-alkycyclobutanones and electron spin resonance (ESR) spectroscopy (3-10). The ESR is a well-established technique for the nondestructive detection of molecules containing unpaired electrons by means of their interaction with an external magnetic field, and has been used to detect the presence of radiation-induced free radicals in biological samples applied to irradiated meat containing bone, for example, chicken(11-15) and fish(16-18), some fresh fruits such as strawberries(19), dried papayas(20), nuts(3), and some crustacea(21-25) such as shrimp, prawn and crevette.

The purposes of this work are to investigate the detection of irradiated meat-containing bones in detail, the effect of irradiation dose on the radical signals and the stability of these signals under a given storage time.

2. Materials and Methods

Chicken, pork and beef were purchased in a local market and wrapped individually with PE (polyethylene) film. Seven samples [chicken (containing leg bones), pork (containing back, rib and leg bones), beef (containing back, rib and leg bones)] of each cut were irradiated with doses of 0, 1, 3, 5 and 7 kGy at ambient temperature using a Co-60 irradiator (point source, 100 kCi) located in the KAERI (Korea Atomic Energy Research Institute, Taejon, Korea). Flesh meat and connective tissue were removed as completely as possible from the bone using a scalpel, and all traces of marrow were removed by scraping in order to avoid the additional free radical species in the marrow. Then each sample was dried in a freeze dryer (Samwon Freezing Engineering Co., Model: SFDSF12) for about 18 hours, from which the suitable sample fragments (about 2.0 mm thick and 3.0 mm long) were removed for analysis. A quartz tube with an internal diameter of about 4.0 mm was packed sufficiently to fill the height of the micro resonator (approximately 2.5 cm). It was then placed in a cavity within the EPR X-band spectrometer (Bruker ESP 300 spectrometer. Germany). The resonant cavity was placed between the poles of a strong electromagnet, which provided the intense magnetic field required. If external magnetic field is applied to such paramagnetic species the unpaired electron can only occupy one of two state : (a) parallel($g_{\parallel}$) to the external field (lower energy state), (b) anti-parallel($g_{\perp}$) to the external field (higher energy state)(26). Electrons can be made to resonate between these two states by the application of microwave energy. Then spectroscopy is the measurement and interpretation of energy differences between the energy levels of the two

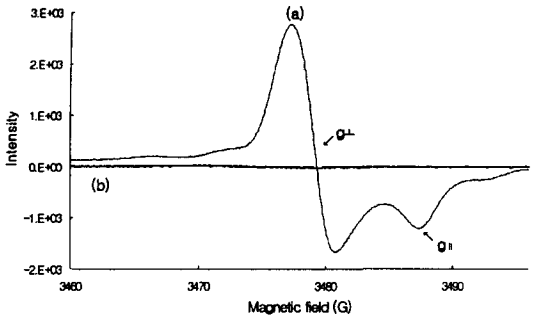

Fig. 1. Characteristic ESR Spectra Derived from (a) Irradiated and (b) Non-irradiated Beef Containing Bone

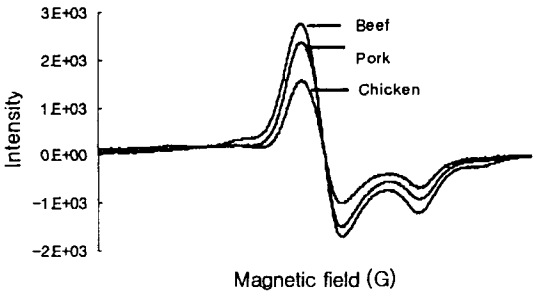

Fig. 2. ESR Signal Responses from Different Irradiated Meats Containing Bone

spin state. The setting of ESR spectrometer were given as follows; field (center field 348 mT, sweep width 50 G), microwave (frequency 9.75 GHz, power 2.997 mW), signal channel (time constant 5.120 ms, sweep time 41.943 s), receiver (receiver gain 1×10^4, modulation frequency 100 KHz, modulation amplitude 2.00 G) and temperature (room temperature).

All signals were normalized to the lowest gain to obtain the dose-response curves. The spectra were double integrated over the magnetic field ranging of 345.5-350.5 mT. The samples irradiated were also measured after 9 weeks at room temperature to determine the stability of the free radicals. Mean values and standard deviations were calculated

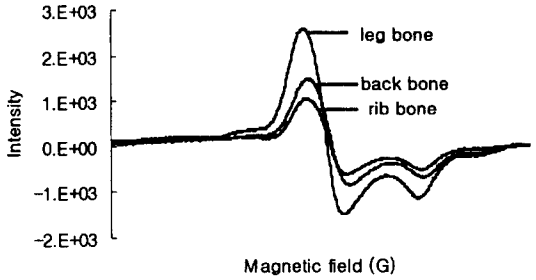

Fig. 3. ESR Responses of Different Cuts from Irradiated Beef Containing Bone

using area of 3 different samples for each irradiation doses.

3. Results and Discussion

An identical characteristic signal and g-factor were observed from all the irradiated meat containing bone used in this work. Two typical ESR spectra for irradiated and non-irradiated (control) samples are shown in Fig.1. When the bone was treated with the ionizing radiation, free radicals were trapped. Irradiated samples were recognized by the appearance of a typical asymmetric signal at 348 mT. The signal of the irradiated bone consisted of two radiation induced lines with $g_{\parallel} = 1.998 \pm 0.00009$ and $g_{\perp} = 2.003 \pm 0.00006$. Non-irradiated bone gave a broad and weak ESR signal due to the bone marrow. The irradiated bone presented an asymmetric absorption curve due to the organic radical (CO_2^-) derived from hydroxyapatite ($Ca(PO_4)_6(OH)_2$), the main constituent of the bone according to other previous studies(27,28).

The signal intensity was the greatest in beef, intermediate in pork and the lowest in chicken bone (Fig. 2). The signal intensity of smaller animals are lower than larger species depending on the calcification status of the bone(29).

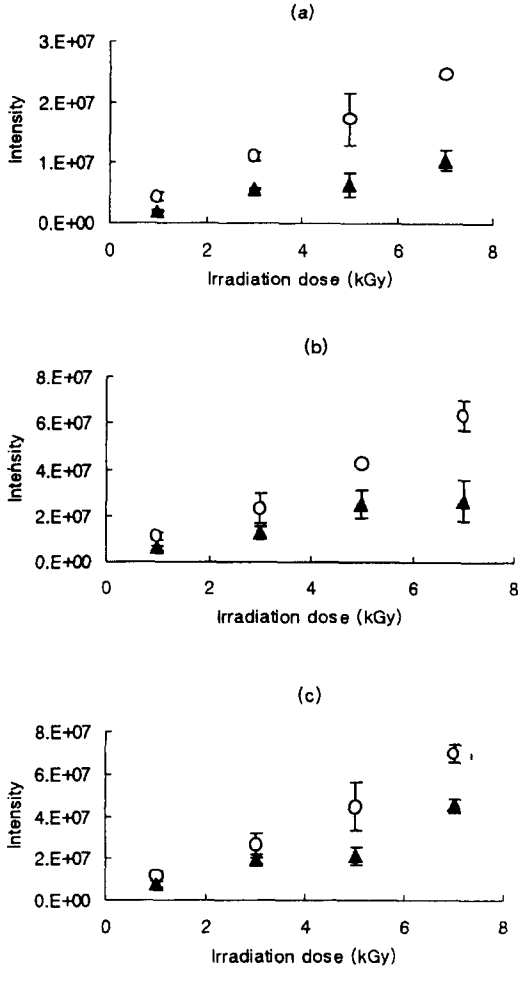

Fig. 4. ESR Signal Changes of (a) Chicken, (b) Pork, and (c) Beef Leg Bone During Storage at Room Temperature(○ : after 0 week, ▲ : after 9 weeks)

However, smaller variations in the signal intensity were observed between the same species. When bones from different cuts of pork and beef were examined, the intensity of the ESR was the greatest in leg bone, intermediate in back bone and lowest in rib bone, which also depended on the calcification status(29). Quantitatively, the harder and more calcified bones gave a greater signal for a given radiation dose (Fig. 3).

The decrease of the ESR signal intensity for the

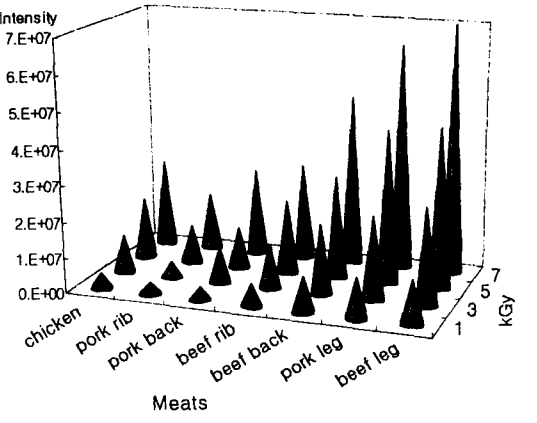

Fig. 5. Dose-dependent ESR Signal Responses in Irradiated Meats Containing Bone

irradiated sample, mean values were calculated using 3 different samples for each irradiation doses, representing the decrease of the ESR signal intensity and the error bars were represented the standard deviations of the mean ESR signal intensity (Fig.4). The signal intensity was decreased after a 9 week storage at room temperature, but was sufficiently remained throughout its expected shelf-life. These findings on the stability of signal intensity are in agreement with other results(30,31). Different irradiation doses produced different signal areas that make possible to estimate the absorbed dose of treated meat (Fig 5). Signal intensity increased linearly with the applied dose (1 to 7 kGy) and agreed well with other published results(31,32). The linear response was possible to quantify the dose received within applied doses. The ESR signal is characteristic of irradiation and provides unequivocal proof that the sample has been irradiated. Thus the ESR spectroscopy has potential for the identification of irradiated meats containing bone such as chicken, pork and beef.

As a consequence, the ESR technique can be used as an identification test of irradiated food such as meats containing bone within the

maximum time of commercial storage.

4. Conclusions

It was possible to differentiate between irradiated and non-irradiated meats containing bone using the ESR spectroscopy and to quantify the dose received within described limits. The ESR spectroscopy turned out a non-destructive and may be reliable method to detect irradiated meats containing bone.

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