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RESEARCH ARTICLE

Development of a Method Based on ESR Spectroscopy for the Identification of Irradiated Beef, Pork, and Chicken Meats

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Abstract The electron spin resonance (ESR) spin-trapping method for the detection of irradiated beef, pork, and chicken was studied using a α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN) spin trapper in the dose of 0.5-7 kGy. Irradiation caused a significant increase in the ESR signal intensity of samples with hyperfine coupling constants of a^N=1.57 mT and a^H=0.25 mT, which correspond to lipid-derived radicals. In contrast, un-irradiated samples exhibited a weak ESR signal with no hyperfine coupling constants. The irradiation-induced lipid radical stability vs. temperature was also studied at room temperature, –4 and –18°C using 3 kGy irradiated beef. Temperature did not affect ESR signal intensity or the hyperfine coupling constants. To investigate the applicability of the proposed procedure for pork and chicken, a comparison of the spectra at the hyperfine coupling constants confirmed the presence of lipid-derived radicals in the samples.

Keywords: electron spin resonance (ESR) spectroscopy, α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN), irradiation, lipid-derived radical

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Introduction

Food irradiation is a technology that can be safely used to eliminate microbial contamination and disinfect insect pests, directly reduce the use of toxic chemicals and extend shelf life (1). The irradiation of food has been studied extensively to improve its safety. Treatment of food with low-dose (<10 kGy) irradiation can kill at least 99.9% of *Salmonella* in poultry and an even higher percentage of *Escherichia coli* O157:H7 (2). The US Food and Drug Administration (FDA) also approved irradiation for poultry and red meats to control food pathogens and extend the product shelf life (3). Although properly irradiated food is safe and wholesome, the irradiation of food has only been permitted at regulated doses in many countries. To facilitate international trade, regulatory authorities need a reliable method to identify irradiated food. Furthermore, consumers should be able to make their own free choice between irradiated and non-irradiated food (4).

The development of analytical methods to detect irradiated food is very important from the point of view of regulation and consumer confidence. Recent research efforts on detection methods for irradiated food are oriented towards the development of sensitive and simplified analytical methods to identify irradiated food (5). In particular, numerous methods have been developed to detect irradiated meats. The analysis of irradiation-induced hydrocarbons and 2-alkylclobutanones can verify whether food containing fat is irradiated or not. European countries have adopted the analysis of hydrocarbons and 2-alkylclobutanones to detect irradiated meats, such as chicken, pork, and eggs (6,7), through organic solvent extraction and separation by a Florisil column. Although irradiated meats are successfully detected by analysis of hydrocarbons and 2-alkyl-clobutanones, this method uses many organic solvent and

is complex and time-consuming.

The detection of free radicals in food matrices by electron spin resonance (ESR) spectroscopy is widely used in the fields of food irradiation and lipid oxidation (8). This method is rapid and simple and can immediately indicate whether or not a food product has undergone a possible irradiation treatment (9). In addition, it provides direct evidence of free radical production in the biological system (10). Thus, we hypothesized that the detection of radiation-induced radicals in lipids and proteins will serve as an adequate method for the detection of irradiated meats. Herein we disclosed an ESR spin-trapping method, which has not been previously reported, using an α-(4-pyridyl-1-oxide)-*N-tert*-butylnitrone (POBN) spin trapper to determine the irradiation status of meat products.

Materials and Methods

Samples and irradiation Meats (beef, pork, and chicken) were purchased from a local market in Jeoungup, Korea. All the reagent-grade chemicals including POBN were purchased from the Sigma-Aldrich (St. Louis, MO, USA). The meats were irradiated at 0.5, 1, 3, and 7 kGy using a ^{60}Co irradiation source at room temperature in the Korea Atomic Energy Research Institute (point source, AECL IR-79; Nordion, Ottawa, Canada). All samples were stored at room temperature, −4 and −18°C after irradiation.

Sample preparation Extraction of the lipid components of meats was performed as previously described (11). Briefly, irradiated meats (1 g) were incubated with 100 mM POBN (1 mL) for 30 min at 0°C. The meats were homogenized in 5 mL of 2:1 chloroform:methanol and 4 mL of deionized water using a homogenizer in an ice bath. A total of 20 mL of 2:1 chloroform:methanol was added to the homogenate, which was shaken and then centrifuged at 850×g for 10 min. The chloroform layer was isolated and dried by passing through a sodium sulfate column. After evaporating the sample, ESR spectra were immediately recorded at room temperature using quartz flat tube in the ESR spectrometer (JES-TE300; Jeol Co., Tokyo, Japan) equipped with tissue cell (LC20).

ESR measurements The ESR spectrum was measured at a microwave frequency of 9.4 GHz, microwave power of 20 mW, modulation amplitude of 0.1 mT, modulation frequency of 100 kHz, conversion time of 0.6 s, sweep width of 10 mT, and time constant of 0.03 s using an ESR spectrometer (JES-TE300; Jeol Co.). At this time, the spectra of samples were scanned to record the signal intensity (peak-to-peak height).

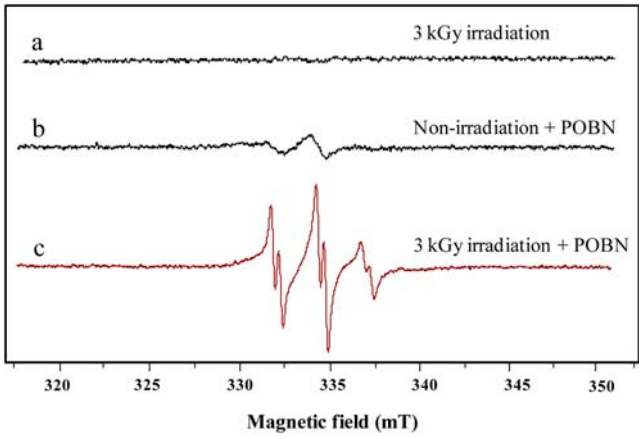

Fig. 1. ESR spectra from 3 kGy irradiated beef samples (a), and non-irradiated (b) and 3 kGy irradiated beef samples incubated in POBN (c).

Results and Discussion

ESR spectra of irradiated beef The development of a method for the determination of whether or not foods have been irradiated must meet several criteria. Apart from the general requirements of low cost, high speed, and applicability to a wide range of food stuffs, the technique should be non-destructive, specific, and combine simplicity with rapid measurement. Therefore, we selected ESR to develop a novel method for the rapid and simple detection of irradiated meats by measuring the level of irradiation-induced lipid radicals. Radical adduct formation was investigated in irradiated beef. Three kGy irradiated beef sample (a) that was not incubated in POBN not exhibited ESR signal (Fig. 1). Un-irradiated beef (b) incubated in POBN exhibited a weak ESR signal with no hyperfine coupling constants (Fig. 1). In contrast, 3 kGy irradiated sample that was incubated in POBN (c) produced ESR signals that corresponded to lipid-derived radicals released from irradiated beef (Fig. 1). The irradiation treatment with POBN significantly increased the signal intensity with hyperfine coupling constants of a^{N}=1.57±0.05 mT and a^{H} =0.25±0.07 mT, similar to those reported for the POBN radical adduct of a carbon-centred, lipid-derived radical (11). The irradiation-induced oxidation of unsaturated fatty acids can be confidently assumed to promote the formation of unpaired-spin-density species that are transformed after their interactions with POBN into the stable radicals displayed in the spectrum (12).

Effect of microwave power on the signal intensity Next, the impact of microwave power on an irradiated beef sample was examined. The microwave field strength was varied from 1 to 26 mW to obtain the progressive

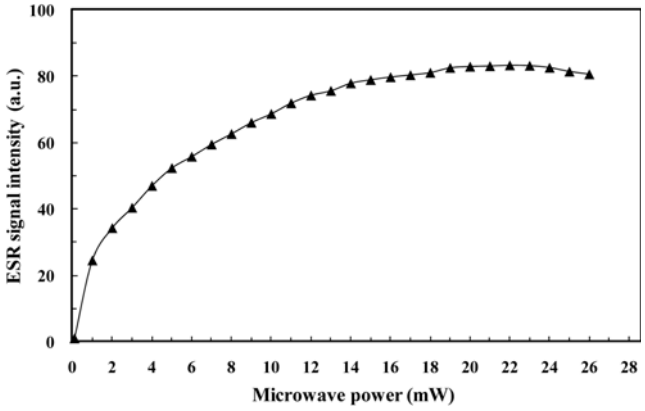

Fig. 2. Effect of microwave power on the signal intensity of 3 kGy irradiated beef samples.

saturation behaviour using 3 kGy irradiated beef (Fig. 2). The effect of saturation was manifested by a continuous non-linear increase of the ESR signal intensity with microwave power, reaching a maximum (22 mW) that was followed by a decrease. The observation is in concordance with a previous study, which reported that the best microwave power to detect lipid-derived radicals is 20.2 mW (13).

Effect of POBN concentration on the signal intensity The impact of POBN concentration to detect lipid-derived radicals was than tested. To determine the appropriate POBN concentration, radical adduct formation using POBN concentrations of 50, 100, and 150 mM was investigated in the 3 kGy irradiated beef. The ESR signal intensity that was recorded for samples untreated and treated with POBN are shown in Fig. 3A. An increase in the POBN concentration was strongly correlated with ESR signal intensity with no change in the hyperfine coupling constants. In the 50 mM POBN treated sample, the intensity of the lines was 23-fold higher than those of the non-treated sample. The relative ratio of the resonance lines was 40 and 47-fold with the 100 and 150 mM POBN treatments, respectively.

Effect of absorbed dose on the signal intensity The ESR signal intensity for the non-irradiated beef and beef irradiated at 0.5, 1, 3, and 7 kGy are shown in Fig. 3b. The non-irradiated beef exhibits a weak ESR signal and does not show any hyperfine coupling constants. In contrast, the signal intensity of the irradiated beef was significantly increased compared with that of the non-irradiated beef. In addition, the irradiated beef had hyperfine coupling constants of a^{N}=1.57±0.05 and a^{H}=0.25±0.07 mT. Importantly, the observation that the hyperfine coupling constants do not change and other lines do not appear in the dose range of 0.5-7 kGy should be emphasised. These results indicate that applying this ESR analysis to the meats at dose above 0.5 kGy should be sufficient for the identification of irradiated meat.

Stability of lipid radicals on the temperature The stability of irradiation-induced lipid radicals in meat is one of the crucial elements for the determination of irradiation. Therefore, radical stability vs. temperature was studied at room temperature, −4 and −18°C using beef samples irradiated at a 3 kGy dose. The variations of the ESR spectra and signal intensities of the beef samples (a and c) are shown in Fig. 4. Temperature did not affect the ESR signal intensity or the hyperfine coupling constants. Importantly, the observation implies that signal intensity and spectra of irradiation-induced lipid radicals decreased during the 3-day storage period but it could be used to identify irradiated samples.

ESR spectra of various meats Pork and chicken samples were also analyzed to investigate the applicability of the proposed procedure. The samples directly incubated with POBN after irradiation, and the lipid-derived radicals were

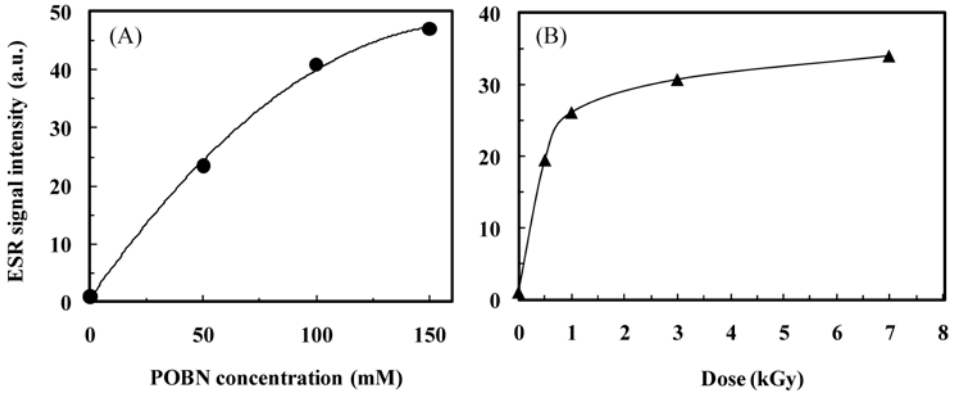

Fig. 3. ESR signal intensity of beef samples as a function of POBN concentration (50, 100, and 150 mM) (A) and of absorbed dose (0.5, 1, 3, and 7 kGy) (B).

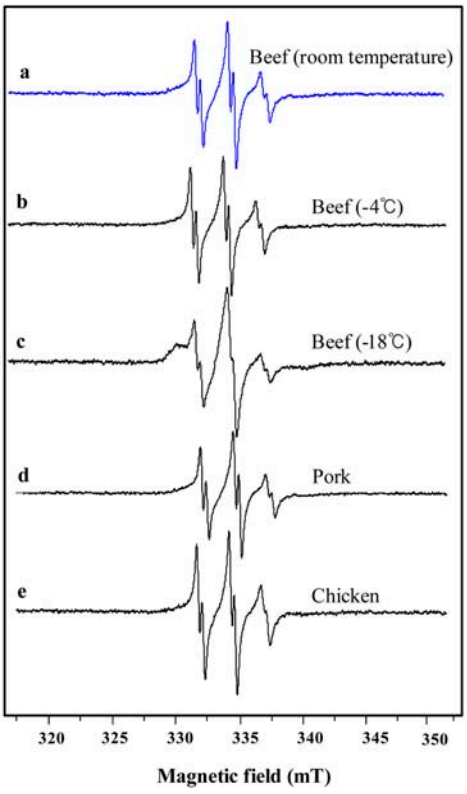

Fig. 4. Variation of ESR spectra from 3 kGy irradiated various samples.

detected by ESR. The ESR spectra corresponding to these samples (d and e) are shown in Fig. 4. A comparison of the spectra at the hyperfine coupling constants confirmed the presence of lipid-derived radicals in the samples, as they completely matched the standard spectrum of beef sample. The ESR spectra displayed a 6-line pattern with hyperfine coupling constants of a^N=1.57 and a^H=0.25 mT.

The present study demonstrated that the ESR spin-trapping method is an excellent alternative for the detection of irradiation treatment on various meats. This method combines a rapid and simple ESR method with the POBN-trapping of irradiation-induced lipid radicals. The irradiation treatment with POBN significantly increased signal intensity, with hyperfine coupling constants of a^N=1.57 and a^H=0.25 mT. In contrast, no spin adduct formation was detected by ESR in the un-irradiated samples. Furthermore, we confirmed the applicability of the proposed procedure for different meats, such as pork and chicken. These samples had similar hyperfine coupling constants under the same conditions. This proposed ESR spin-trapping method with POBN is simple and rapid and enables the determination of whether or not various meats have been irradiated.

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