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Identification of Irradiated Crabs by ESR Spectrometry

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ABSTRACT - Electron spin resonance (ESR) spectroscopy was used to investigate the effect of irradiation dose on the ESR signal intensity of irradiated crabs and the stability of these radicals under 9 weeks of storage. Swimming and small crabs were irradiated with doses of 0, 1, 3, 5 and 7 kGy using a Co-60 irradiator at ambient temperature. A claw, a walking leg and a carapace of the crab pieced and dried were placed in a resonant quart tube within an EPR X-band spectrometer. The irradiated crabs presented an asymmetric absorption in shape at g_1=2.002± 0.003 and g_2=1.998±0.005, and were different from the non-irradiated ones. The intensity of the ESR signals was greatest in the claw, intermediate in the carapace and lowest in the walking leg. Samples given low and high doses of irradiation could also be distinguished. The ESR signal after irradiation was stable, even after a 9-week storage.

Key words □ ESR spectroscopy, Signal intensity, Signal stability, Swimming and small crab

Introduction

Crustacea are susceptible to bad handling and poor quality control, which are associated with microbial contamination, especially *Salmonella* and *Vibrio*. These microorganisms may present a public health hazard if not inhibited or destroyed. The treatment of crustacea with ionizing radiation will reduce the numbers of potential pathogens and spoliage organisms, thus giving a microbiologically safer product with a longer shelf-life at chill temperatures.

In some countries, such as the Netherlands and the United Kingdom, there are regulations about which kinds of products are eligible for the ionizing radiation treatment and what is maximum dose. In other countries, however, the treatment is prohibited by law. Therefore clear, unambiguous labeling and enforcement of the labeling claims through a reliable detection tests would assist consumer acceptance of the process.

Like other processing technologies, irradiation imparts energy to food and produces free radicals, which generally have such a short life span that they cannot be detected. However, if a food contains hard, dry components, such as bone, shell or seeds, the radicals can be trapped and their presence confirmed by ESR spectroscopy.[1,2] The purpose of this work is to investigate the detection of irradiated crabs in detail; the effect of irradiation dose on the radical signals and the stability of these signals under a given storage time by ESR spectroscopy.

Materials and Methods

Swimming and small crabs were purchased from a local market and wrapped individually with PE (polyethylene) film. Each crab was 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 KAERI (Korea Atomic Energy Research Institute, Taejon). After irradiation the meat and connective tissues were removed as completely as possible from the crabs using a scalpel. Then each claw, walking leg and carapace was divided and dried in a freeze dryer for about 18 hours. From these small sample fragments (about 1.0 mm width and 1.0 mm length) 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

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resonator (about 0.1 g). It was then placed in the cavity within the EPR X-band spectrometer (Bruker Win-EPR spectrometer, Germany). The resonant cavity was placed between the poles of a strong electromagnet, which provided the intense magnetic field required. The settings of the ESR spectrometer were given as follows; field (center field 344 mT, sweep width 1000 G), microwave (frequency 9.70 GHz, power 6.34 mW), signal channel (time constant 2.560 ms, sweep time 20.97 s), receiver (receiver gain 4.48 10^4, modulation frequency 100 KHz, modulation amplitude 4.00 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 300-390 mT. The samples irradiated were also measured after 9 weeks to determine the stability of the free radicals.

Results and Discussion

The ESR signals derived from non-irradiated and irradiated samples were characteristic of Mn^{2+} with six equally spaced resonance peak[3] and the signals of the irradiated crabs consisted of two radiation induced lines with g_1=2.002±0.0003 and g_2=1.998±0.005(Fig. 1). An asymmetric absorption curve is due to the trapped radicals (CO^{2-}, CO_3^{3-}) derived from chitin, a structural polysaccharide composed of N-acetyl glucosamine, which is a major component of the cuticle matrix.[4,5]

Calcium may also have contributed to the radiation induced signal.[5,6,7] It was this additional peak which is an indication of irradiation in the crabs and could potentially be used to identify irradiated samples qualitatively.

Differing signal intensities have also been reported for chicken bone excised from different sites within the carcass.[8,9] In the case of bone, at least part of the difference has been attributed to the degree of crystallinity of the individual bones. When ESR signals from different cuts of crab were examined, the intensity of the ESR was greatest in the claw, intermediate in the carapace and lowest in the walking leg (Fig. 2). In crabs the claws are the most fragile appendage and perhaps differences in the structure composition may affect the components ability to trap radicals.

Fig. 3 shows the decrease of the ESR signal intensity for an irradiated sample, representing the mean values calculated using 3 different samples for each irradiation dose, and error bars representing the standard derivations of the mean ESR signal intensity. The signal induced by 1 kGy and 3 kGy doses exhibited a greater stability compared to claws given higher doses (5 kGy and 7 kGy). The signal intensity was sufficiently stable after a 9 week storage at 5°C. The ESR signal from crabs could persist over an extended period, similar to other results.[10,11]

Results obtained from different radiation doses to

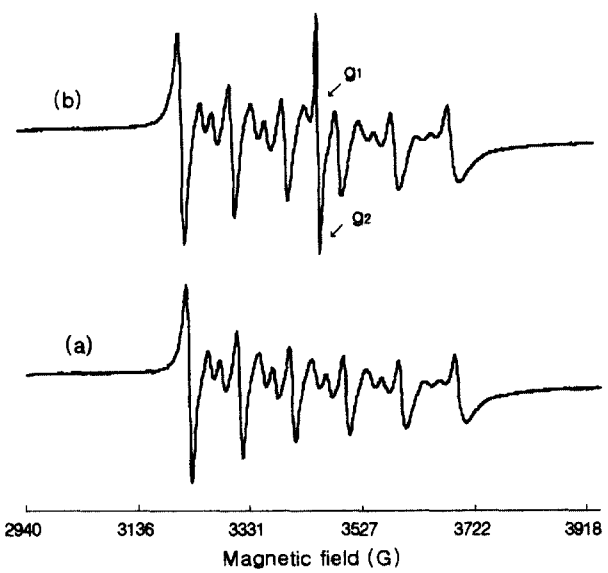

Fig. 1. Characteristic ESR spectra derived from (a) non-irradiated and (b) irradiated crabs.

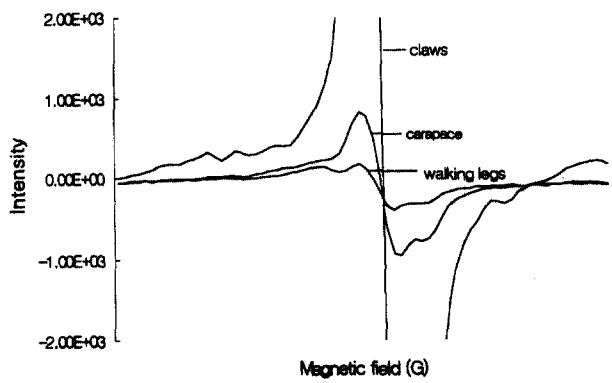

Fig. 2. ESR signal responses from different parts in irradiated crabs.

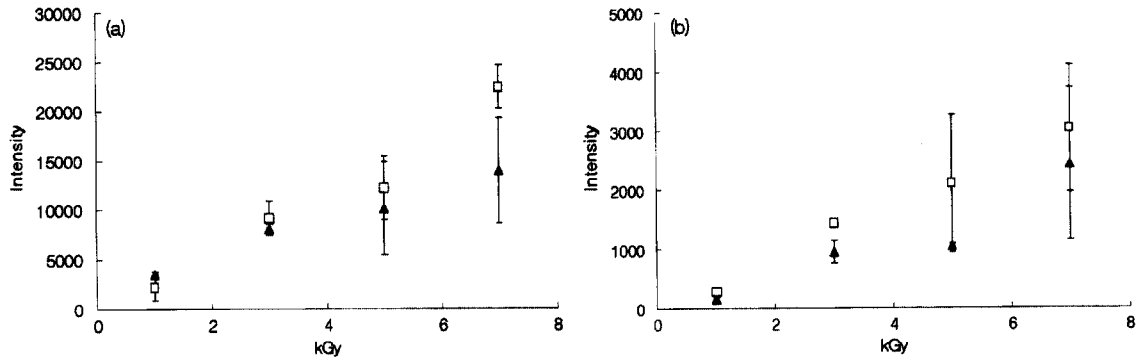

Fig. 3. ESR signal decrease of (a) swimming crab's claw and (b) small crab's carapace during storage (□: after 0 week, ▲: after 9 weeks)

Table 1. ESR signal heights in irradiated crabs (arbitrary unit)

		1 kGy	3 kGy	5 kGy	7 kGy
Swimming crab	claw	2,180±1,331[1)]	9,183±1,686	12,204±3,226	22,415±2,229
	walking leg	42±138	644±131	789±167	1,192±198
	carapace	185±74	1,119±107	1,584±560	2,021±362
Small crab	claw	3,056±872	9,697±1,291	14,276±685	13,854±2,146
	walking leg	24±4	100±92	266±101	421±192
	carapace	283±60	1,445±7	2,102±1,156	3,041±1,075

[1)]Mean±SD

estimate the irradiation dose by measuring signal height were presented in Table 1. Signal intensity of all samples increased linearly with applied doses (1 kGy-7 kGy) with the exception of the small crabs claw at 7 kGy, which was probably due to saturation of the free radicals sites within the small crabs claw. Thus ESR spectroscopy has potential for the identification of irradiated crab within the maximum commercial storage time (6 months).

Conclusion

It has been possible to differentiate between irradiated and non-irradiated crabs using ESR spectroscopy and to quantify the dose received within the commercially irradiated doses. The technique of ESR spectroscopy has proved to be a non-destructive technique and currently provides a specific method for the detection of irradiated crab.

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국문요약

ESR spectroscopy를 이용하여 게류의 방사선 조사 유무와 선량관계 및 EAR 신호의 안정성에 관하여 알아 보았다. 꽃게와 방게를 0, 1, 3, 5, 그리고 7 kGy로 방사선을 조사한 후 살을 제거하고 집게다리, 등 일반다리의 겉 표피만을 취해 건조시킨 다음, Burker Win-EPR spectroscopy를 이용하여 신호의 특성을 알아보았다. 방사선 조사된 꽃게와 방게 시료는 g_1=2.002±0.0003, g_2=1.998±0.0005에서 특유의 비대칭 신호를 나타내어 비조사 시료와 뚜렷하게 구별되었으며, 각 시료의 부위별 비교는 꽃게와 방게에서는 똑같이 집게다리>등>일반다리의 순으로 신호의 차이가 나타났다. 조사선량의 증가에 따른 ESR신호의 높이는 직선적으로 증가하였다. 또

한 이들 신호의 크기는 5℃에서 9 주간의 저장기간에서도 안전하여 방사선 조사 유무의 판별은 장기간의 저장에서도 가능하였다. 따라서 ESR spectroscopy를 이용한 방사선 조사 게류의 검지법은 빠르고 확실하며 준정량적인 방법임을 알 수 있었다.

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