

Investigation of Different Factors Affecting the Electron Spin Resonance-based Characterization of Gamma-irradiated Fresh, White, and Red Ginseng

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Fresh (raw roots), white (dried), and red (steamed-dried) ginseng samples were gamma-irradiated at 0 to 7 kGy. Electron spin resonance (ESR) technique was used to characterize the irradiation status of the samples, targeting the radiation-induced cellulose radicals after different sample pretreatments. All non-irradiated samples exhibited a single central signal ($g=2.006$), whose intensity showed significant increase upon irradiation. The ESR spectra from the radiation-induced cellulose radicals, with two side peaks ($g=2.0201$ and $g=1.9851$) equally spaced (± 3 mT) from the central signal, were also observed in the irradiated samples. The core sample analyzed after alcoholic-extraction produced the best results for irradiated fresh ginseng samples. In the case of irradiated white and red ginseng samples, the central (natural) and radiation-induced (two-side peaks corresponding to cellulose radical) signal intensities showed little improvement on alcoholic-extraction. The water-washing step minimized the effect of Mn^{2+} , but reduced the intensity of side peaks making them difficult to identify. The effect of different origins was negligible, however harvesting year showed a clear effect on radiation-induced ESR signals.

Keywords: *Panax ginseng*, Irradiation, Identification, Electron spin resonance

INTRODUCTION

Food irradiation is now permitted in more than 55 countries for hundreds of different raw and processed food products to enhance hygienic quality, extend shelf-life, and reduce risk of food-borne diseases. Health safety concerns have been properly addressed through intensive research of many decades resulting endorsement from major health authorities. However, general consensus over this wholesome technology requires delivery of scientific based information to the consumer. Considering consumer right of choice and regulatory requirements, proper labeling is mandatory for irradiated products [1].

The genus *Panax* (Araliaceae family) consists of more than 10 species which are most famous and important

medicinal plants. The root of *Panax ginseng* Meyer, mainly cultivated in Korea and Northeast China, has been heat-processed to improve its medicinal efficacy in Korea [2,3].

Ginseng is a medicinal herb and part of a different functional food in far-eastern Asian countries. It is usually available in the form of red ginseng (steamed and dried), dried white ginseng roots and powder, extracts, tea, and liquid drinks. However, unprocessed raw roots are also available. Likewise other herbs, ginseng and ginseng products have potential risk of microbial and insect attack and require an effective sterilization technique with the least compromise on quality attributes [4,5].

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Different scientists reported notable results for the improvement of hygienic quality of ginseng products using irradiation treatment [4,6]. The Korean Food and Drug Administration has approved irradiation of ginseng (maximum dose 7 kGy) [7]. *Panax ginseng* has solid international market, which may be enhanced by providing better quality products using irradiation [6]. However, for international trade of irradiated products, reliable identification methods have key importance [8].

Electron spin resonance (ESR) analysis is an effective technique to characterize irradiated food on the basis of radiation-induced radicals. Kwon et al. [5] and Nakamura et al. [8] reported effective identification of red ginseng using ESR spectroscopy however; limited studies are available in the case of raw ginseng roots and white dried ginseng. Especially raw ginseng roots need detailed ESR study after effective sample drying technique due to their high moisture content. In literature, freeze-drying and alcoholic-extraction methods are reported for the ESR analysis of fresh products with moisture content [9,10]. Delincee and Soika [11] reported the improvement in radiation-induced ESR signal of fresh and dried products using alcoholic-extraction. Recently, we observed the improved ESR signal in the identification of irradiated sauces by including the water washing step [12].

In this study, improved ESR-based identification of radiation-induced radicals was attempted using different sample pretreatments. The results were compared and discussed to develop better ESR-based identification method for different ginseng products. Effect of different origin and age at the time of harvesting on radiation-induced ESR signals was also investigated.

MATERIALS AND METHODS

Samples and irradiation

Fresh ginseng, white ginseng (dried), red ginseng (dried and powdered) were cultivated in Punggi and Geumsan regions of South Korea and harvested at the age of 4 to 6 years. The samples were irradiated (0, 1, 4, and 7 kGy) using a Co-60 gamma-ray source (Dose rate 1.5 kGy/h; AECL, IR-79, MDS Nordion International, Ottawa, ON, Canada) at the Korean Atomic Energy Research Institute, in Jeongeup, Korea. Alanine dosimeters with a diameter of 5 mm (Bruker Instruments, Rheinstetten, Germany) were used to confirm the applied dose, and the free-radical signals were measured by a Bruker EMS 104 EPR analyzer (Bruker Instruments). After treatment, all samples were stored at room temperature.

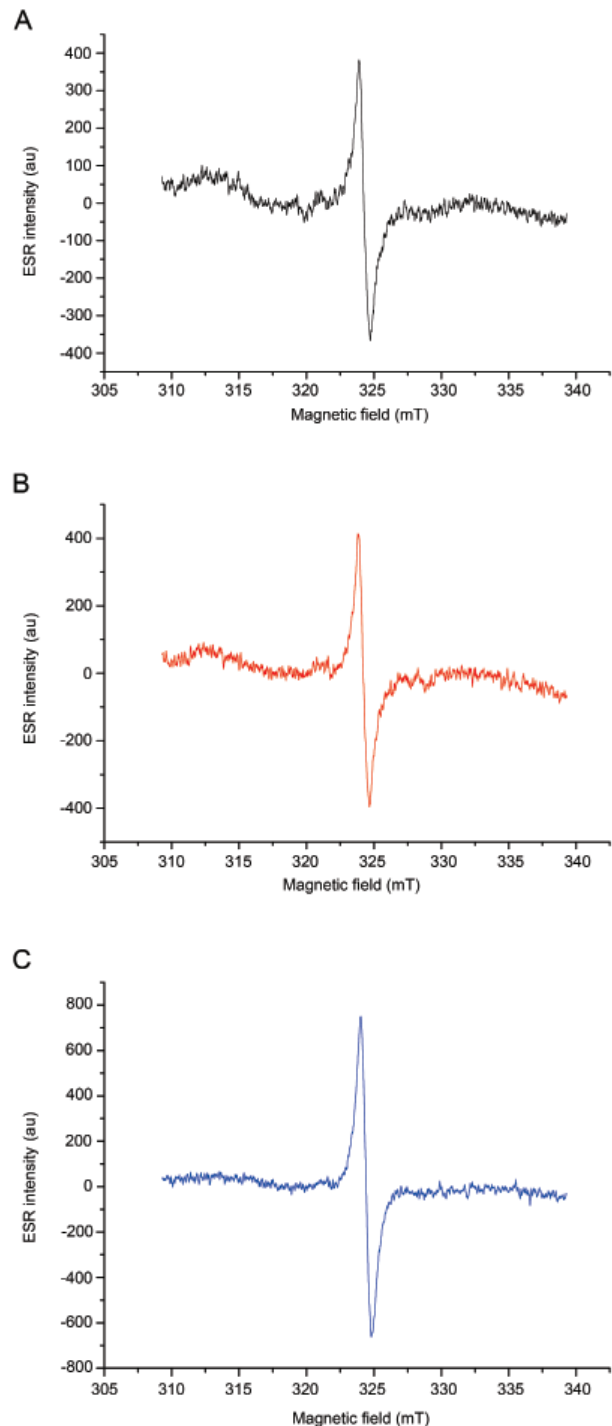


Fig. 1. Electron spin resonance (ESR) spectra of non-irradiated fresh ginseng using different sample drying pre-treatments. (A) Freeze-drying, (B) alcoholic-extraction, (C) water washing and alcoholic-extraction.

Electron spin resonance spectroscopy

Three different sample pretreatments were employed before ESR measurements: 1) FD: freeze-drying (Bondiro; Ilsin Bio Base, Yangju, Korea) of all samples [10].

Table 1. Measuring conditions for the electron spin resonance analysis of the sauces after different sample pretreatments

Specification		Condition
Microwave	Power	9.10-9.11 GHz
	Frequency	0.4 mW
Magnetic field	Center field	324.5±1 mT
	Sweep width	10-25 mT
Signal channel	Modulation frequency	100 kHz
	Modulation width	1-2 mT
	Amplitude	50-400
	Time constant	0.03 s
	Sweep time	30 s
Temperature	Ambient temperature	

2) AD: alcoholic-extraction of fresh roots, dried white and red ginseng samples to reduce moisture content and improve ESR signals as described by Delincee and Soika [11]. 3) WAD: mixing (20 min) of ground/powdered sample with distilled water and insoluble residues were used after alcoholic-extraction as described above.

Approximately 0.1 g of the pulverized (<1 mm) sample was placed in a quartz ESR tube (5 mm dia.). The tube was then sealed with a plastic film, and stored in the dark in a desiccator at 40±5% relative humidity. ESR signals were measured as described in the European standard (EN 1787, 2000). The X-band ESR spectrometer (JES-TE 300; Jeol Co., Tokyo, Japan) was used at room temperature under the conditions given in Table 1. Mea-

surements were performed three times ($n=3$), and mean values (±standard deviation) were reported. The results were analyzed using Microsoft Excel (Microsoft Office 2010 package; Microsoft, Redmond, WA, USA) and Origin ver. 8 (Microcal Software, Northampton, MA, USA).

RESULTS AND DISCUSSION

Spectral features of non-irradiated samples

A single central signal ($g=2.0040$) was observed in all nonirradiated samples irrespective of sample type and pretreatment (Fig. 1). Various researchers also reported similar central signals in different foods of plant origins [13-18] and were attributed to organic (semiquinone) radicals [13,19-21]. Upon different sample pretreatments, the intensity of this signal was the lowest in alcoholic extracted samples while the highest in water treated samples, however qualitative appearance was the same without any change in g -value ($g=2.0040$). The central signal intensity was similar in sample of different origins, however variable results were observed for samples harvested at different ages of the plant.

Spectral features of irradiated samples

Upon irradiation, all samples showed a dose-dependent increase in the intensity of the central signal. The radiation-induced two side peaks ($g=2.0201$ and $g=1.9851$) started appearing in all 4 kGy-irradiated samples, which were most prominent in 7 kGy-irradiated samples. The

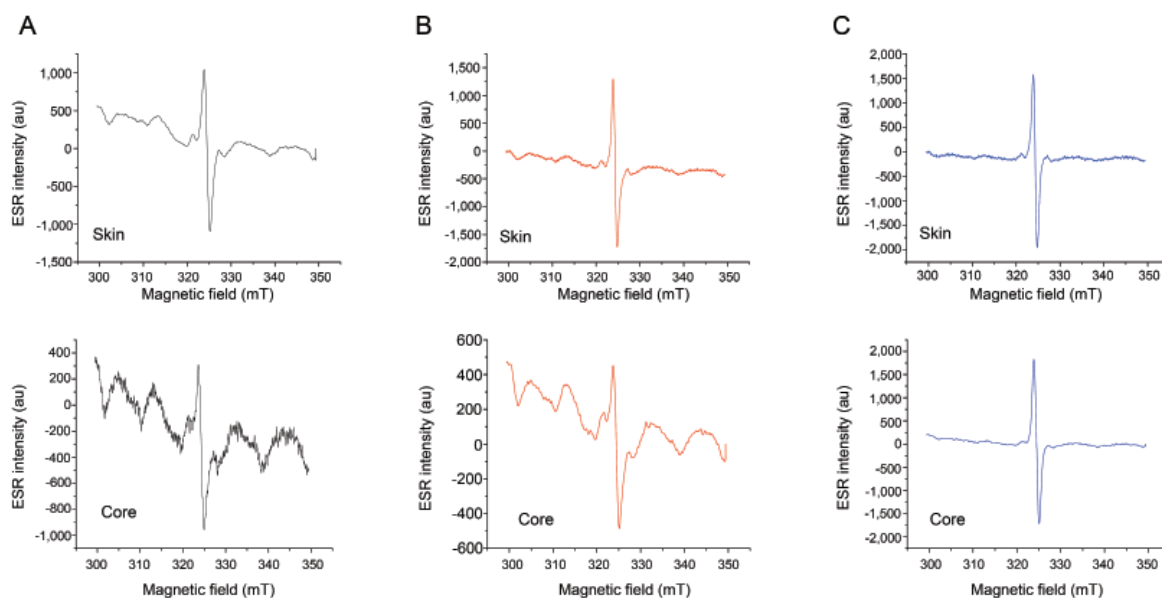


Fig. 2. Electron spin resonance (ESR) spectra of 7 kGy-irradiated fresh ginseng using different sample drying pre-treatments. (A) Freeze-drying, (B) alcoholic-extraction, (C) water washing and alcoholic-extraction.

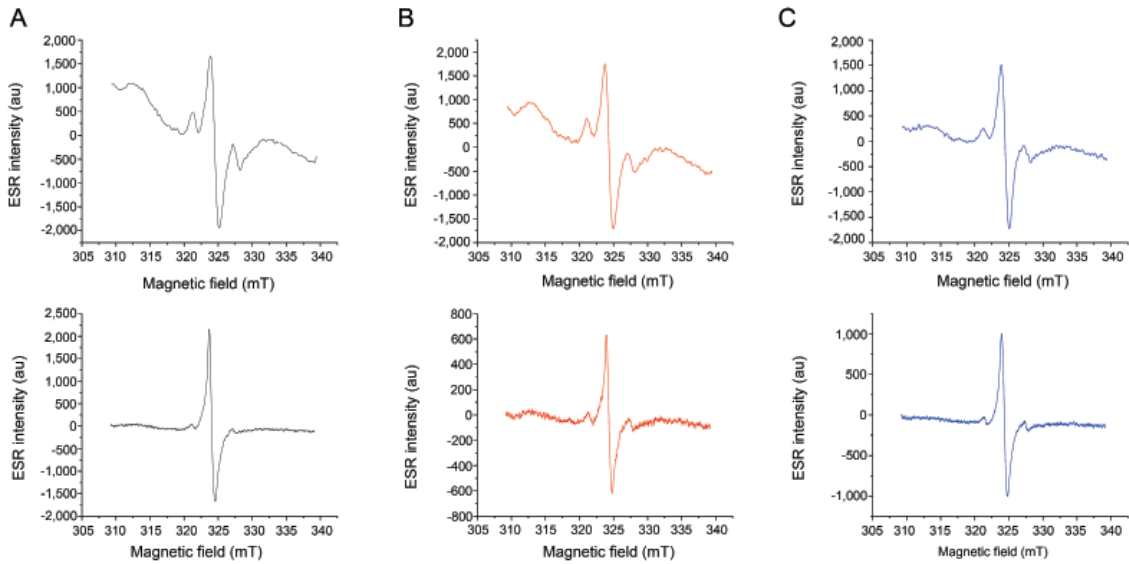


Fig. 3. Electron spin resonance (ESR) spectra of 7 kGy-irradiated white ginseng (above) and red ginseng powder (below) using different sample pretreatments. (A) Freeze-drying, (B) alcoholic-extraction, (C) water washing and alcoholic-extraction.

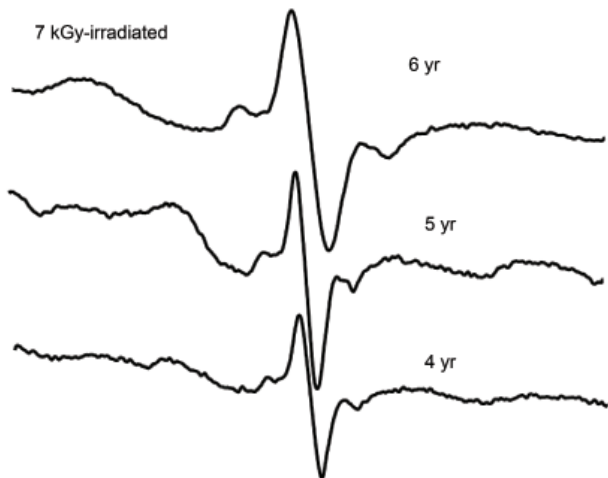


Fig. 4. Electron spin resonance spectral characteristics of 7 kGy-irradiated fresh ginseng harvested at different plant age.

radiation-induced side peaks were equally spaced at about ± 3 mT from the main signal and were associated with the radicals produced by irradiation in cellulose-containing foods [22]. Different drying techniques (FD, AD, and WAD) were employed to get improved intensity of side peaks with minimum effect of Mn^{2+} , resulting in easy and clear identification of irradiation treatment [23]. In case of fresh ginseng samples (Fig. 2), results were most promising with clear side peaks and the lowest effects of Mn^{2+} when core samples were used for the ESR analysis after alcoholic-extraction (AD). However, comparable results were observed in the case of FD and

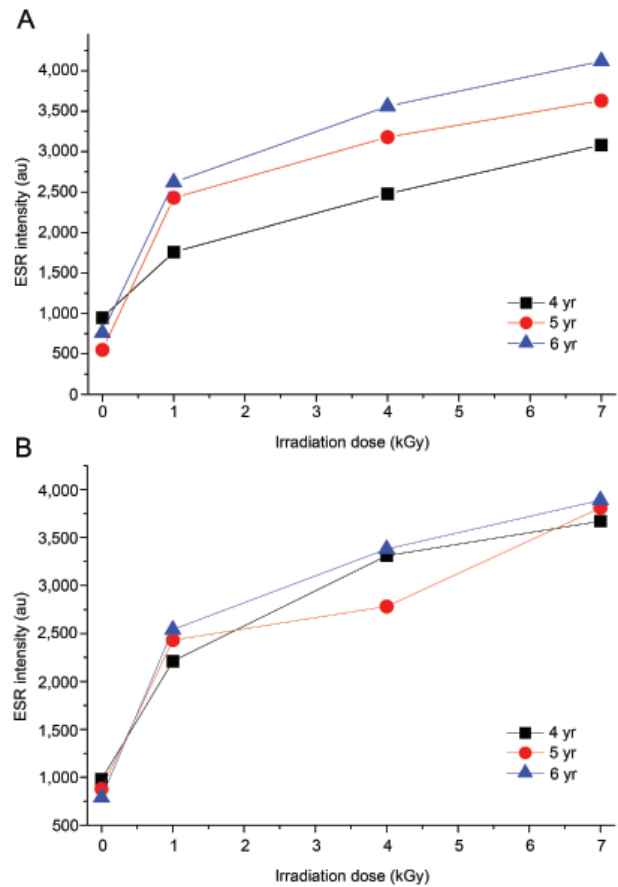


Fig. 5. Effect of plant age at the time of harvesting on electron spin resonance signal intensity of irradiated fresh ginseng of different origins. (A) Harvested from Punggi, (B) harvest from Geumsan.

AD samples for both skin and core samples (Fig. 2). WAD samples showed a high intensity of central signal but results were not as valuable as the side peaks were not clear. In dried white ginseng and red ginseng powder (Fig. 3), alcoholic-extraction provided better signal quality compared to FD samples. The effect of alcoholic-extraction was most clear in red ginseng samples with clear side signals. However, the signal appearance was similar in all studied samples where the lowest intensity of side peaks was recorded in WAD samples. The distance (g_1 - $g_2=6.033\pm 0.057$ mT) and g -values ($g_1=2.0242\pm 0.0003$ and $g_2=1.9866\pm 0.0003$) of two side peaks did not vary considerably regarding the change in the sample type and pretreatments. The same trend was found by Jesus et al. [9] in fruit pulp samples with different sample pretreatments.

The samples of different origins provided similar qualitative and quantitative results. However, there was a general increasing trend in ESR intensity of harvested irradiated samples aged at 4, 5, and 6 years except 4 kGy-irradiated sample of Geumsan origin harvested in year 4 (Figs. 4 and 5).

In conclusion, the non-irradiated samples provided the single central signal whose intensity increased upon irradiation with the emergence of two side peaks of radiation-induced cellulose radicals. In fresh ginseng samples, alcoholic-extraction provided better ESR signals especially when the ginseng core was used for the analysis. In dried samples, little improvement was observed upon alcoholic treatment. However, the effect of Mn^{2+} was reduced that made signals more clear. Samples of different origins provided similar results, however the age at harvest showed effects on radiation-induced ESR signal.

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