

Identification of fish irradiation by EPR spectrometry: signal intensity and stability

Ibragim Medzhidov*, Nailya Vasileva, Irina Polyakova, and Natalya Sanzharova

NRC «Kurchatov Institute» - RIRAE, Obninsk, Russian Federation

Abstract. In this work, the application of electron paramagnetic resonance spectroscopy for assessing the irradiation of fish is described. Pink salmon was chosen as the research object and irradiated at 3 and 6 kGy doses. A linear correlation was observed between the increase in EPR signal intensity and the radiation dose. It was demonstrated that the fish used in this study had previously been irradiated by the supplier for microbiological sterilization. To assess the signal decay kinetics, repeat spectroscopy of samples was conducted over the course of a year after irradiation. A decrease in EPR signal intensity is observed initially after irradiation, attributed to the decay of short-lived radicals. The results indicate that EPR spectroscopy is an accurate and sensitive way to determine whether fish has been irradiated.

1 Introduction

In recent years, cases of foodborne pathogens have made food safety a top public health priority. Due to logistical problems during transportation and maintaining temperature conditions, fish losses are a significant issue. Bacterial activity is the primary cause of spoilage [1]. According to the latest FAO data, approximately 35% of fish products are lost due to microbiological spoilage. Various methods for microbial inactivation are actively used to prevent spoilage. In addition to traditional methods, ionizing radiation is widely recognized as an effective control measure for inactivating pathogenic bacteria [2-8].

Several authors [9-13] have reported the high effectiveness of microbial inactivation, targeting bacteria such as *Staphylococcus spp.*, *Listeria spp.*, *Enterobacteriales spp.*, *Vibrio spp.*, *Salmonella spp.*, and others. However, the degree of inactivation depends on various internal (e.g., spore formation, radiation sensitivity) and external (e.g., temperature, oxygen presence, processing technology) factors [14]. In addition to the sterilization effect, ionizing radiation processing also extends the shelf life of products. For example, the authors of the study [15] observed an increase in storage time of fish when exposed to a dose of 2 and 3 kGy and stored at a temperature of 1-2 °C. In fact, they reported an extension of 11 days in comparison to the control sample.

Following the requirements for food irradiation established by the Codex Alimentarius is crucial. Exceeding the radiation dose leads to a disruption in the quality of the product, while an insufficient radiation dose may not be as effective.

* Corresponding author: immedzhidov@mail.ru

However, irradiating food products can induce changes in the chemical structure of the products. It is necessary to adhere to norms and standards. The Codex Alimentarius also prohibits re-irradiation, as it leads to food degradation and spoilage. Therefore, quality control of the products is crucial to ensure food safety. The European Committee for Standardization in Brussels has approved ten positively tested methods as sufficiently sensitive and reliable. Among them, three primary methods are thermoluminescent spectrometry, photoluminescent spectrometry, and electron paramagnetic resonance spectrometry.

Thermoluminescent spectrometry (TL) is a method of measuring the light emission released during the heating of the irradiated material. The method operates on the principle that when the product is irradiated, electrons in the substance become excited and move to higher energy levels. Upon subsequent heating, the excited electrons release their energy through light flashes and return to a stable state. The instrument records the intensity of light flashes, which can be used to determine whether food products have been irradiated. The intensity of light flashes is proportional to the amount of energy absorbed during irradiation. This technique is regulated by the European Standard EN 1788:2002.

Photoluminescent spectrometry (PSL) is similar to TL, but it uses optical stimulation to release the energy of excited electrons instead of heating. This technique is regulated by the European Standard EN 13751:2009.

Electron Paramagnetic Resonance (EPR) is a method for studying materials with unpaired electrons. Under ionizing radiation, free radicals, which act as paramagnetic species, are formed in biological substrates. Identification occurs through the magnetic field absorption by the research object and the generation of quantum transitions between energy levels. The EPR signal's intensity is typically maintained for several months, making it possible to accurately determine radiation treatment while storing food products and raw materials. Moreover, the EPR method has a high degree of reliability.

The method is regulated by numerous Russian (GOST R 52529-2006, GOST 31652-2012, GOST 31672-2012, etc.) and European (EN 1787:2000, EN 13708:2001, etc.) normative documents.

The main objective of this study was to explore the potential use of EPR spectrometry for detecting fish irradiation and analysing the decay kinetics of the signal over time.

2 Materials and methods

2.1 Fish samples

Pink salmon (from the salmon family) caught in the Pacific Ocean's Far Eastern Russian region was used for this study. The fish were stored at a temperature not exceeding -18 °C. Eight months after catching, the fish were purchased from a supplier for further investigation.

2.2 Irradiation and sample preparation

The fish was divided into several parts, and small bone tissue samples were taken from each part. The samples underwent a meticulous cleaning process, where muscle, tendons, membranes, and bone marrow were delicately removed using a medical scalpel (in accordance with the Russian Standard GOST R 52529–2006). The cleaned samples were then placed in an isothermal container with refreezable gel-type packs and sent for irradiation. The GUR-120 gamma facility (NRC «Kurchatov Institute» — RIRAE, Obninsk, Russian Federation) was used for ionizing radiation treatment with a ⁶⁰Co source. Samples were exposed to doses of 3 and 6 kGy at a temperature not exceeding -18 °C. A non-irradiated

sample was taken as a control. The dose rate was 1.5 kGy/h. Dosimetry procedures were carried out in accordance with the Russian Standards GOST 34155-2017 and GOST 8.664-2019.

After irradiation, the samples were dried in a dry heat oven at 40 °C for 20 hours. They were then kept at room temperature for 60 minutes before being ground into < 0.5 mm fragments. The prepared samples (50 mg each) were placed in 5 mm diameter quartz tubes for EPR analysis.

2.3 EPR-spectroscopy

EPR spectra were recorded at room temperature (≈ 23 °C) using an ESR70-03 XD/2 spectrometer (Minsk, Belarus) operating at the X-band microwave frequency (9.4 GHz). All samples were positioned identically in the spectrometer. The height of the substances in the tubes was equal for all samples. The procedure was repeated three times, and the registration process was carried out in accordance with the Russian Standard GOST R 52529–2006. Spectroscopy of the samples was carried out over the course of a year after irradiation. The decay kinetics of the free radicals was determined by changing the intensity of the signal at various time intervals.

The parameters of the EPR spectrometer during registration were as follows:

- central field – 3350 Oe;
- field range – 1000 Oe;
- modulation frequency – 100 kHz;
- modulation – 4 Oe;
- microwave power – 50 mW;
- gain ratio – 50;
- recording time – 700 s.

2.4 G-factor calculation

The g-factor is a dimensionless quantity that describes the magnetic moment of a particle or molecule when exposed to an external magnetic field [16]. It is a critical parameter in EPR spectroscopy to determine the electron spin and the nature of the studied paramagnetic species.

The g-factor can be calculated using the following formula [16]:

$$h\nu = g\mu_B B \quad (1)$$

where h is the Planck constant, ν is the microwave frequency of the X-band, μ_B is the Bohr magneton, and B is the selected magnetic field point (T).

3 Results and discussion

3.1 EPR spectrum registration

The results obtained from the EPR spectroscopy of pink salmon bone tissue samples are shown in Figure 1. There is an observed increase in signal intensity with an increase in radiation dose. The signal intensity was determined by measuring the amplitude of the first derivative of the absorption peak using the software's built-in function.

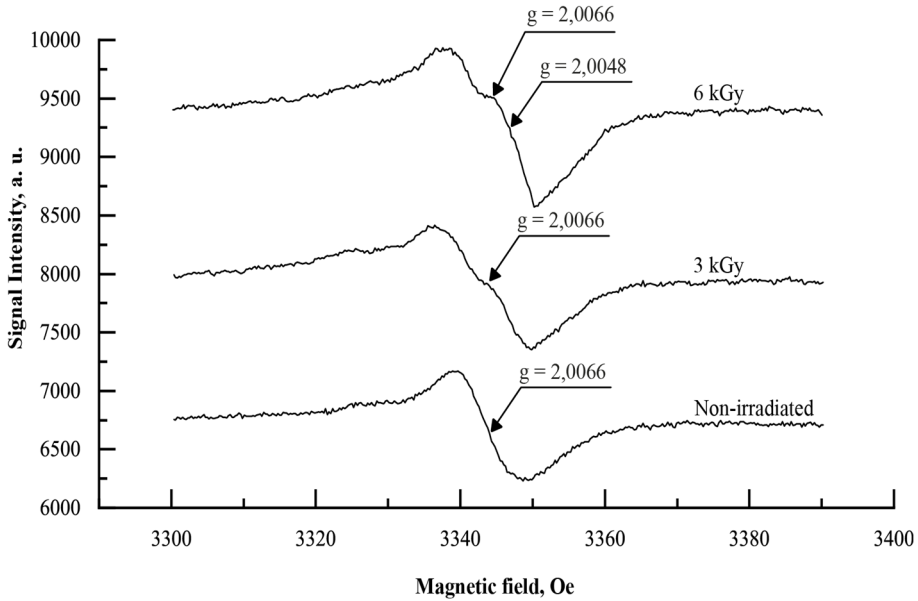


Fig. 1. EPR spectra of pink salmon bone tissue subjected to irradiation at doses of 3 and 6 kGy, compared to the control sample.

The results showed that the signal intensity was 1350 a.u. for the 6 kGy sample, 1060 a.u. for the 3 kGy sample, and 930 a.u. for the non-irradiated sample. In irradiated samples, additional magnetic centres are formed with a g-factor equal to 2,0066. The fish-bone tissue contains a significant amount of incomplete proteins and mineral components, of which about 80% is calcium phosphate. Therefore, the absorption in the samples was primarily attributed to the significant components of calcium, such as calcium apatite – hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$. The control sample also has a clear signal, slightly less intense than irradiated samples. Further clarification from the supplier revealed that the fish had previously been irradiated after being caught. However, the type of source used for the irradiation is currently unknown. EPR spectroscopy was conducted on freshwater fish caught under home conditions to confirm this information.

3.2 Comparison of non-irradiated samples of silver carp and pink salmon

A silver carp was selected for comparison. After being caught, it was stored at a temperature not exceeding $-18\text{ }^\circ\text{C}$. Bone tissue was extracted from the thawed fish. The sample preparation and EPR spectrum registration were similar to the sample preparation for pink salmon. The obtained results are presented in Figure 2.

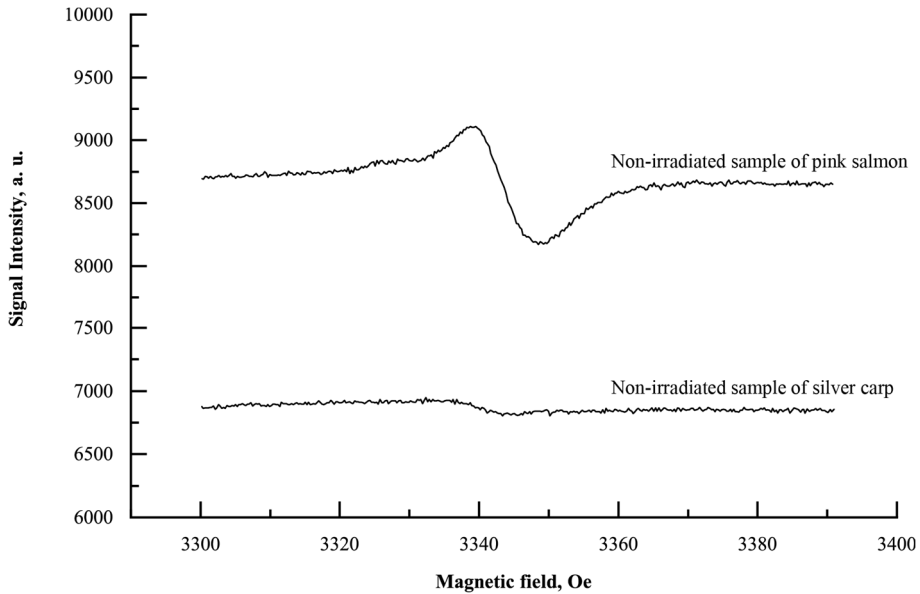


Fig. 2. EPR spectra of non-irradiated bone tissue samples of pink salmon and silver carp.

The control samples show a significant difference in signal intensity: 930 a.u. for pink salmon and 110 a.u. for silver carp. This comparison verifies that the fish had been irradiated by the supplier after being caught. It is worth noting that about 8 months had passed since the fish were caught, and the beginning of our research indicates the possibility of identifying the fact of fish irradiation after 8 months, at least. Some authors [17-19] have also reported either the absence or low content of free radicals in non-irradiated fish samples. Nevertheless, even with repeated irradiation, a linear correlation is observed, showing an increase in signal intensity with increasing radiation dose.

3.3 Study of the EPR signal decay kinetics

The stability of induced free radicals in sample matrices is one of the crucial elements for retrospective dose assessment. Unfortunately, free radicals tend to decay, hindering accurate EPR analysis. Typically, the decrease in signal intensity over time occurs due to the recombination of particles with other paramagnetic particles and their transformation into another paramagnetic molecule. This is the main barrier that prevents spectroscopy from being conducted after a long period following irradiation. However, the signal decay process is individual for each substance. Some materials are able to induce stable radicals, allowing irradiation detection even after several years, for example, sugar [20]. Therefore, investigating the decay kinetics of the EPR signal over time is crucial.

For this study, bone tissue samples were measured on the spectrometer at 10 days, 1, 2, 4, 7, and 12 months after irradiation. The tubes with the substance were stored in a closed room without light at atmospheric pressure. The substance was not removed from the tubes and was kept in the original tube. The samples were placed in the spectrometer at the same level for all samples. The results are presented in Figure 3.

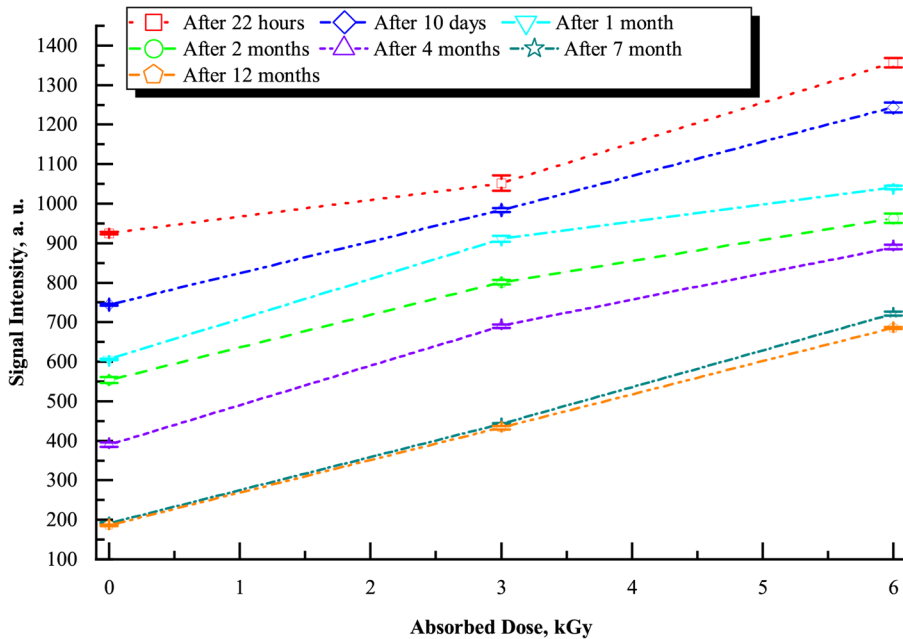


Fig. 3. The decay kinetics of the EPR signal over the course of a year after irradiation.

A signal attenuation is observed over time for both irradiated and non-irradiated samples. Storage conditions directly affect the attenuation of the signal. In this study, the samples were stored in a dried state at atmospheric pressure and room temperature after irradiation, which may have affected the signal decay. Further research is planned to confirm or refute this theory, taking into account the above factors. A decrease in the intensity of the control sample is also observed. Based on our assumptions, there are two primary factors that contribute to the issue. The first factor is the presence of free radicals which were generated from the irradiation processing by the supplier. The second factor is the storage conditions. The maximum signal attenuation is observed in the first time after irradiation, as a large number of short-lived radicals are formed after irradiation. Nevertheless, although EPR signal attenuation is observed, it is possible to identify whether the fish was irradiated after a certain period of time based on the data obtained.

4 Conclusion

The purpose of this study is to explore the potential of EPR spectrometry in determining whether fish has been irradiated, as well as its ability to detect a signal over a specific period of time. A regular increase in the signal intensity with increasing radiation dose was observed. The g-factor value ranged from 2.0048 to 2.0066. It was demonstrated that the investigated fish had previously been irradiated by the supplier. Re-irradiation also induces the formation of free radicals, but identification becomes more complicated.

To study the kinetics of the EPR signal attenuation, the samples were re-registered over the course of a year. A decrease in signal intensity was observed during the initial period after irradiation, as short-lived radicals decayed first.

Based on the obtained data, it can be concluded that EPR spectrometry is a promising physical method that allows for numerous measurements to be conducted. It is also suitable for identifying whether fish has been irradiated. Currently, this method is actively being implemented in most countries for border control of the safety of imported products. EPR

spectrometry differs from existing analogues in its high reliability of results, ease of sample preparation, and fast signal registration.

References

1. H.H. Huss, *Quality and quality changes in fresh fish* (FAO Fisheries Technical Paper; No. 348, Rome, 1995)
2. J. Park, J. Ha, *Int. J. Food Microbiol* **289**, 127-133 (2019)
3. M.T. Munir, M. Federighi, *Foods* **9**, 878 (2020)
4. S. Shahi, R. Khorvash, M. Goli, S.M. Ranjbaran, A. Najarian, A. Mohammadi Nafchi, *Food Science & Nutrition* **9**, 5883-5896 (2021)
5. B. Bisht, P. Bhatnagar, P. Gururani, et al., *Trends Food Sci. Technol.* **114**, 372-385 (2021)
6. X. Fan, B. Niemira, *Food Safety Engineering* (2020)
7. O. Odueke, K. Farag, R. Baines, S. Chadd, *Food and Bioprocess Technology* **9**, 751-767 (2016)
8. A. Prakash, *Radiation Physics and Chemistry* **129**, 50-52 (2016)
9. H. Zhang, W. Zhou, *Advances in Food and Nutrition Research* **100**, 287-328 (2022)
10. T.M. Osaili, A.A. Al-Nabulsi, T.F. Aljaafreh, *Int. J. Food Microbiol.* **278**, 20-25 (2018)
11. H. Zhang, H. L. Seck, W. Zhou, *Innovative Food Sci. Emerg. Technol.* **67**, 102556 (2021)
12. E.F.E. Mohamed, A.E.-S.E. Hafez, H.G. Seadawy, et al., *Microorganisms* **11**, 1105 (2023)
13. D. Apaydin, G. Tirpanci Sivri, A.Ş Demirci, *Food Science and Technology International* (2023)
14. D. W. Thayer, *Proceedings Food Irradiation* **99**, 12-14 (1999)
15. I. Ahmed, M. Alur, A. Kamat, J. Bandekar, P. Thomas, *International Journal of Food Science & Technology* **32**, 325-332 (1997)
16. G. R. Eaton, S. S. Eaton, L. J. Berliner, *Electron Paramagnetic Resonance: A Practitioner's Toolkit* (Wiley, New York, NY, USA, 2010)
17. R. Timakova, S. Tikhonov, N. Tikhonova, V. Poznyakovskiy, *Foods and Raw materials* **5**, 162-169 (2017)
18. W. Stachowicz, G. Strzelczak-Burlinska, J. Michalik, A. Wojtowicz, A. Dziedzic-Goclawska, K. Ostrowski, *J. Sci. Food Agric.* **58**, 407-415 (1992)
19. A. Paari, P. Kanmani, R. Satishkumar, N. Yuvaraj, V. Pattukumar, M. Agrawal, V. Arul, *Journal of Food Processing and Preservation* **36**, 152-160 (2012)
20. I. Medzhidov, I. Polyakova, S. Gorbatov, et al., *Herald of the Bauman Moscow State Technical University, Series Natural Sciences* **4**, 141-158 (2023)