The ESR Method to Detect the Irradiated Fish and Beef Bones

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Summary: Electron spin resonance (ESR) spectroscopy was employed for identification and quantification of the more stable free radicals generated by irradiation in bones of fish and beef. The samples were irradiated 1- 5 kGy with gamma rays using Co-60 source. ESR spectroscopy was employed to detect irradiation in samples of fish and beef bones. Results revealed that the irradiated samples showed strong *asymmetric* signals with g_1 2.002 ± 0.001 and g_2 1.998 ± 0.001 respectively, which was a clear indication of irradiation. The control samples were not irradiated and showed typical low intensity *symmetric* signals at g-value of 2.005 ± 0.001 revealing the absence of radiation treatment. It was also observed that intensities of signals due to hydroxyapatite radicals for 5 kGy were normally higher than 1 kGy radiation dose, showing the dose dependency.

Introduction

The use of ionizing radiation in food processing like other commercial processes such as freezing, canning and drying, produces no significant reduction in the nutritional quality of the protein, lipid, carbohydrate and mineral constituents. The Council for Agricultural Science and Technology [1] estimated that a dose of 1 kGy would break fewer than 10 chemical bonds for every ten million bonds present. Cooking, or applying infrared radiation to foods, produces similar changes in chemical bonds. Recently irradiation of meat has emerged as a new and effective preservation technique. The primary purpose of irradiating meats is to kill microbial pathogens and infectious parasites [2]. The technology has the additional benefits of reducing the numbers of spoilage microorganisms, resulting in extended shelf life and retaining their raw appearance as well as quality.

Although properly irradiated foods are safe and wholesome, the consumer should be able to make his own free choice between irradiated and non-irradiated food. When ionizing radiation passes through the food stuffs, free radicals are produced. Most of the free radicals are unstable and combine with neighbouring molecules to give stable products, but some are more persistent and exist for longer period of time in the hard portion of the foods. Even the trapped radicals remain stable for at least 10 years [3]. The existence of these free radicals or paramagnetic centres can be detected with help of ESR in hard parts of the foods without pre-treatment of the sample [4]. ESR measurements were proposed as a practical method for detection of radiation in processed foods [5].

Although the technique is limited to dry and hard portions of food it has been found useful due to the relative ease at which radiation induced ESR signals could be measured in meat, bone, seafood, spices, vegetables, herbs, condiments, fruits and dry fruits *etc.* [6]. In case of foods containing bones, the irradiated samples are recognised by the appearance of a typical asymmetric signal, which is attributed to the trapped radicals in hydroxyapatite, which are produced by irradiation of bones [7].

In view of the potential of food irradiation in Pakistan, testing of the available methods to identify irradiated food is necessary.

Results and Discussion

The results regarding the ESR spectroscopy showing the peak intensities and "g" values calculated for each tested sample are given in Table-1. The unirradiated samples showed, weak typical symmetrical signals with g-value of 2.005 ± 0.001 and are presented graphically in Figs. 1 and 2. All the irradiated samples showed strong asymmetric signals with g_1 2.002 ± 0.001 and g_2 1.998 ± 0.001. This asymmetry in signals was the clear indication of irradiation. The ESR spectra for unirradiated and irradiated samples of beef bones (1 and 5 kGy) are shown in Fig. 1. By comparing the intensities of

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Table-1: Peak Intensity and g-Value in ESR Spectra of Beef and Fish Bones.

Samples	Intensity (nm)		g-Values		
	Unirradiated	Irradiated	Unirradiated	Irradiated	
			g	g_1	g_2
beef	14	23	2.005±0.001	2.002±0.001	1.998
fish	6	68	2.004±0.001	2.00±0.0011	1.998

Irradiation dose = 5.0 kGy

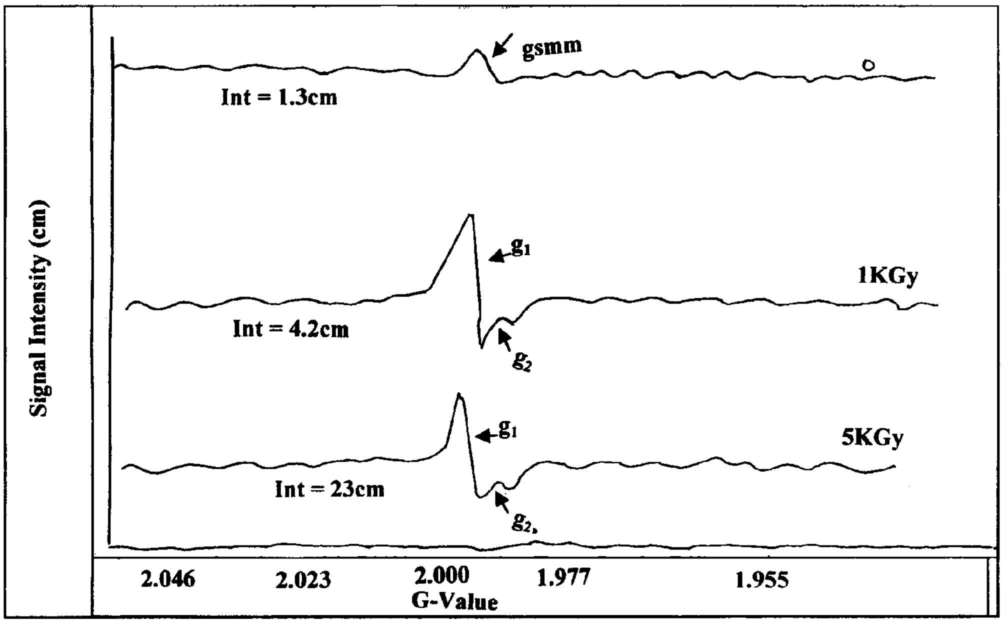

Fig. 1: ESR Spectrum of beef bones.

specific signals, one can get some idea about applied radiation doses. In addition, g-values of the asymmetric signals could also be used for qualitative identification of radiation treatment. The g-values obtained in this case were similar to the typical values, which have been reported earlier for the asymmetric signal from irradiated bone samples [8]. Therefore, from the results of this study, it can be concluded that detection of radiation treatment of beef is possible for the applied doses due to the appearance of asymmetric ESR signals and their g-values.

The differentiation between irradiated and unirradiated fish samples was also possible on basis of g-values as indicated in spectra of fish sample (Fig. 2). The g-values observed in this case were g_0 2.004 ± 0.001 for unirradiated samples and g_1 2.001 ± 0.001 and g_2 1.998 ± 0.001 for 1kGy and 5kGy irradiated samples respectively. In addition, measurement of intensities of the specific ESR signals due to hydroxyapatite-based radicals can give some information about the different doses of radiation applied to fish samples. Similar results have been reported for blue jack mackerel fish, where it was mentioned that weak unspecific signals in controlled samples of the fish become dominant at very low doses and in this way, they contribute towards a lower detectability limit [9].

The analysis of ESR spectra for the fish and beef samples, irradiated by different doses of gamma irradiation showed that intensities of specific signals were dependent upon the applied doses (Fig. 3). Here dose-to-dose variations are easily recognizable with respect to the concentrations of hydroxyapatite radicals present in different irradiated samples. It was observed that intensities of signals due to hydroxyl-

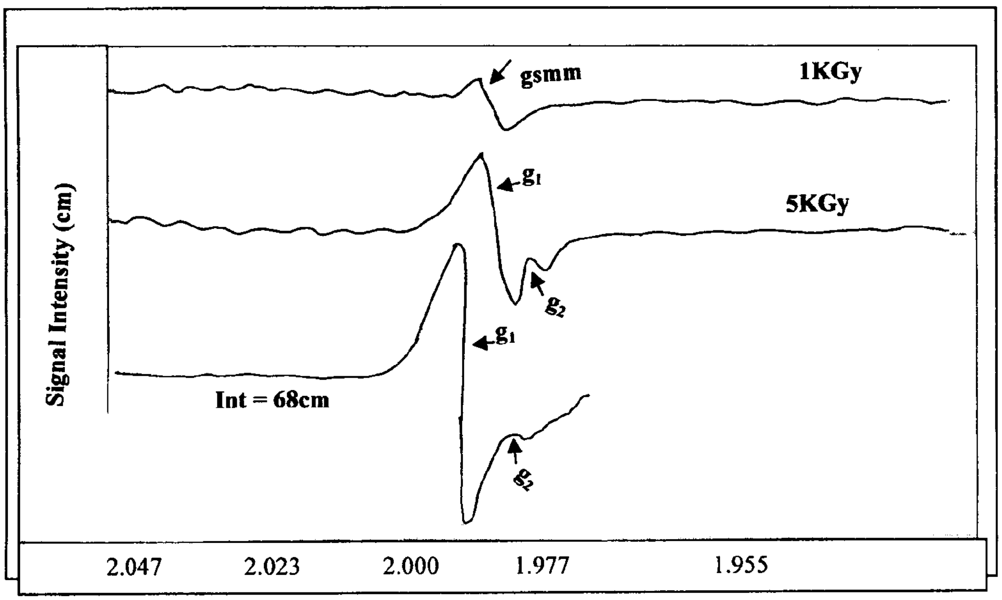

Fig. 2: ESR Spectrum of fish bones.

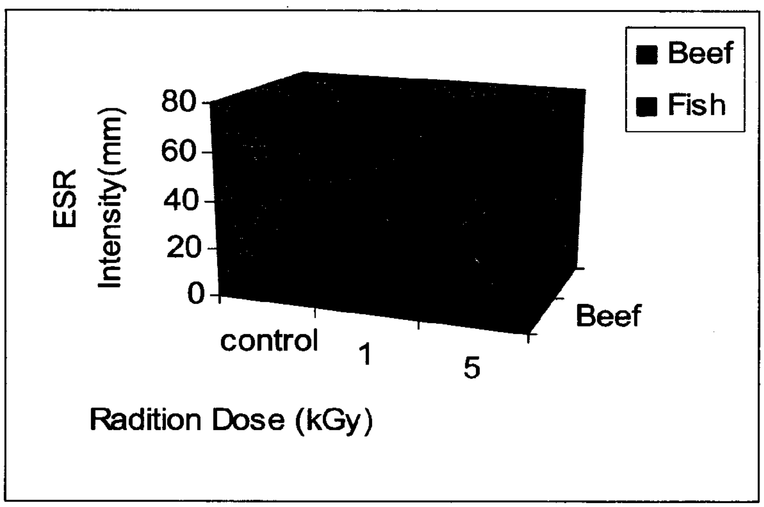

Fig. 3. Dependency of ESR signals intensity with Radiation Doses.

apatite radicals for 5 kGy were higher than for 1 kGy, showing the dependence of intensities upon dose. Therefore, it can be concluded that discrimination between unirradiated and irradiated samples of fish is possible by ESR method and the method can be used for the identification of radiation treatment of the fish and beef.

The present results were in agreement with European standard EN 1786 [10]. This kind of linear relationship between applied radiation doses and intensities of asymmetric signals has also been reported in food stuffs for the applied doses up to 20-25 kGy in some earlier studies [11]. Electron spin resonance appears effective for detecting irradiated bone-containing food and possibly shellfish [12, 13]. Similar results have been observed in earlier studies that hydroxyapatite and organic signals overlapped to some extent in case of low applied doses of radiation. However, these signals could be distinguished spectroscopically because of their distinct nature [14]. In case of samples irradiated by different doses, the intensities of specific signals were prominent for high doses (3 and 4 kGy) has also been reported earlier, where it has also been mentioned that in fish it was not possible to remove all the marrow from bone samples due to the low rigidity and crystallinity of bone [15]. Similarly, according to [16] the combination in the ESR spectra observed in irradiated bone samples was due to 60 % of hydroxyl-apatite with most of the remaining organic fraction in the form of collagen. Some workers [17] worked on fruits and their seeds and found that ESR is reliable in detecting irradiation treatment. While detecting irradiated crustaceans by ESR some scientist [18] pointed out that ESR signal may vary due to species and age of crustaceans.

It is concluded from the results that ESR method is very suitable and can be applied to the varieties of the food materials having hard parts like bones. This technique could easily be applied to distinguish between irradiated and unirradiated food material.

Experimental

Samples of beef and fish were obtained from local market of Peshawar. The samples were washed and packed in transparent polyethylene pouches, and were irradiated to dose levels of I, 2, 3, 4, and 5 kGy with ^{60}Co gamma radiation source, ISSLEDOVATEL (CIS) installed at NIFA, keeping unirradiated controls in each case. The bone samples were ground and analyzed to detect the post irradiation changes employing electron spin resonance spectroscopy on a Jeol ESR spectrometer under the following instrumental conditions:

Magnetic field	3371 $\pm$ 250 Gauss
Modulation of magnetic field	1.6 Gauss
Amplification	3.2×10^{-2}
Scan rate (Sweep time)	16 min/360 mm
Micro-wave power	0.8 mW
Micro-wave frequency	9.44 G. Hz

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