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Irradiated Hazelnut (*Corylus avellana*): Identification and Dose Assessment Using Electron Paramagnetic Resonance Spectroscopy †

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Abstract: Background: Food irradiation aims to eliminate biohazards such as pathogens, microbes, fungi, etc. identification of irradiated food and dose assessment ensures its safe use. Hazelnut is the most universal widespread nut and can be found as a whole fruit or as ingredient in many food types. Objective: Electron paramagnetic resonance (EPR) was used to identify irradiated hazelnut and to assess radiation doses delivered to it using fractions of its kernel and/or shells. Methods: In this paper, parameters affecting the proper detection and evaluation of irradiated hazelnut kernels and shells are studied and analyzed including the response to Cs-137 gamma rays, the effect of the change in microwave power and modulation amplitude values during EPR spectra acquisition and the time dependence of the induced radicals. Results: The stability study of the radiation-induced radicals suggests that it is better to perform EPR measurements for irradiated hazelnut during the first month following irradiation.

Keywords: radiation dosimetry; hazelnut; Electron Spin Resonance; Electron Paramagnetic Resonance; food irradiation

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1. Introduction

Innovative technologies have recently arisen to guarantee the quality and safety of diverse food items in response to the rising worldwide need for food safety and preservation [1–3]. One such technology is the use of ionizing radiation for the preservation of food, which has drawn much attention due to its potential to increase shelf-life and to control dangerous pathogens [3,4]. Irradiated nuts have, however, come under close investigation about their safety and appropriateness for human consumption. In order to investigate the effects of ionizing radiation on nuts, scientists have turned to Electron Paramagnetic Resonance (EPR) spectroscopy [5–8].

Electron paramagnetic resonance (EPR) utilizes the unpaired electrons resulted from irradiation to identify the irradiated food. EPR is an accurate and non-destructive method for the evaluation of radiation doses delivered during irradiation of irradiated food goods, assuring the safety and quality of those products [9]. Through EPR spectroscopy, these free radicals may be identified and measured, providing a window into the probable alterations brought on by radiation [10,11].

The European Committee for Standardization (CEN) has approved EPR as a standard technique for the detection of irradiated food where it is considered in the European Standards EN 1787:2000 (for cellulose), EN13708:2001 (for crystalline sugar), and EN 1786:2001 (for bone) [12], the Codex Alimentarius Commission has ratified the European Standards [13,14]. According to the European standard, all cellulose containing food, even unirradiated samples, exhibit a single (central) signal in their EPR spectra at about g = 2.004, the genesis of the paramagnetic species has been the subject of various theories [11,15,16].

Hazelnut (Filbert) is a rich source of proteins, carbohydrates, unsaturated fatty acids, vitamins and essential minerals. Irradiation of hazelnut ensures the purity of possible pathogen and undesired microbes. [17]. It is of great importance to identify the irradiated hazelnut, especially in cases of the absence of outer shells with a speed test; this helps the global hazelnut trade and ensures safe consumption.

This study aims to employ the EPR technique to identify irradiated hazelnut looking forward to finding a method for the evaluation of radiation doses delivered to hazelnut shell and kernel.

2. Instruments, Materials, and Methods

2.1. Radiation Source and Radiation Dose Determination

Gamma radiation exposure was carried out by utilizing a Cesium-137 gamma source, model GB-150, created by the Atomic Energy of Canada Limited in April 1970, initially possessing an activity of 1000 curies. The measurement and assessment of the air kerma (K_{air}) were conducted following the guidelines outlined in the International Atomic Energy Agency (IAEA) code of practice TRS-(381). This assessment of K_{air} was executed using the secondary standard dosimetry system established by the National Institute of Standards (NIS) in Egypt. This system was calibrated at the Bureau International des Poids et Mesures (BIPM). The values of K_{air} were evaluated, with an associated expanded uncertainty of approximately 0.9% at a 95% confidence level (with a coverage factor of 2).

Additionally, exposure to high radiation doses was carried out utilizing a Cobalt-60 gamma-source (housed within an irradiation cell) situated at the National Center for Radiation Research and Technology (NCRRT), part of the Egyptian Atomic Energy Authority. The radioactive source possessed an activity level of approximately 50,000 Ci, and the rate of dose delivery was approximately 4.0 kGy/h at the location where the samples were placed for irradiation. This irradiation process occurred at room temperature and utilized Perspex phantom irradiation capsules. The radiation doses delivered to the samples ranged from 0.5 to 10 kGy.

2.2. EPR Spectrometer

The electron paramagnetic resonance (EPR) setup employed in this study is EMX-BRUKER system from Germany. This system is outfitted with a 9.5 GHz microwave (X-band) Gunn-Oscillator Bridge with an automatic tuning feature. The configuration utilizes a rectangular resonator, specifically the 4102 ST cavity, which operates in the TE102 mode.

2.3. Hazelnut

Hazelnut (Corylus avellana) is the most popular nut over the world especially in the Mediterranean region where it can be used alone or as food additive. Corylus avellana is of the species Corylus, family of Betulaceae order Fagales and clade Rosids. Hazelnut fruit is composed of a soft kernel covered by a thin brown layer and lays inside a hard-smooth inedible outer shell as shown schematically in Figure 1.

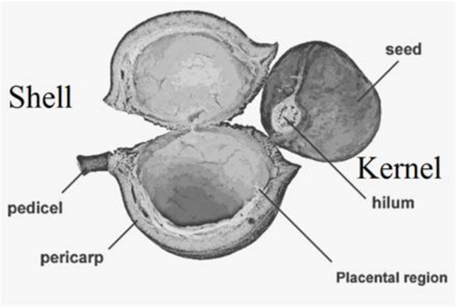

Figure 1. Hazelnut Major structural features.

2.4. Sample Preparation

Shells of hazelnut were crushed gently until they become in the form of fine grains, and kernels were extracted. Pedicel parts were not included in the sample; also, fibers were removed from the placental region of the shell. Regarding the kernels, they underwent a mechanical peeling process to remove their outer skin, followed by the extraction of the hilum portions. Subsequently, the inner flesh of the kernels was extracted and finely divided into small fragments. These small kernel pieces were then placed on a clean cellulose sheet (paper) for approximately 30 min in order to facilitate the removal of fatty substances.

2.5. Evaluation Method

The EPR measurements for hazelnut shells and kernels were conducted with the following parameters: a microwave power of 2.012 mW, a central field set at 344.8 mT, a sweep width of 50.0 mT, a time constant of 163.84 ms, and a conversion time of 81.92 ms. These parameters resulted in 1024 data points per spectrum, and the entire sweep took approximately 84 s. The modulation amplitudes were 1.9 mT for shells and 0.8 mT for kernels, with a modulation frequency of 100 kHz. To ensure signal accuracy, empty tube spectra were recorded prior to recording the spectra of the hazelnut samples.

The acquired EPR spectra were calibrated to the peak-to-peak amplitude of a reference standard material (DPPH). This standard's EPR spectra were recorded before and after each hazelnut sample spectrum to account for variations in the spectrometer's sensitivity. The hazelnut shell samples weighed 0.08 ± 0.014 g, while the kernel samples weighed 0.07 ± 0.016 g. The intensity of EPR signals from each sample was normalized based on its mass. Each individual sample's EPR spectrum was recorded three or more in successive scans, each consisting of a single scan (n = 1) [18,19].

Uncertainties associated to the delivered radiation doses were evaluated according to the ISO guide for the expression of uncertainties [20].

3. Results and Discussion

3.1. Spectral Features

Figure 2 represents three EPR spectra; (a) represents the EPR spectrum of an unirradiated hazelnut kernel, where it appears to have multiple numerous overlapped unresolved signals close to each other and forming a broad hump-like structure. These unresolved signals might be attributed to a number of fatty acid radicals existing natively in kernels of hazelnut. Spectrum (b) is of a 10 kGy gamma-irradiated hazelnut kernel, where a singlet (S_b) can be observed at gb = 2.00981, and Peak-to-peak line width (WPP) of g_b ~ 1.0 mT, this signal might be attributed to radicals of the polyene type which are highly stable at different values of temperature; it was used as a dosimetric signal for irradiated kernels. On contrast to the unirradiated hazelnut kernel, unirradiated hazelnut shell shows no distinctive feature. On the other hand, irradiation of hazelnut shells possesses a sharp singlet (S_c) as can be seen in spectrum (c) which represents the irradiated (10 kGy) external shell of hazelnut, g_c = 2.00979. Peak-to-peak line width (W_{PP}) of g_c is approximately ~ 0.8 mT. The singlet labeled as "g_c" seems to be prevalent in the EPR spectra of various irradiated nuts like walnuts, pistachios, and peanuts. This occurrence might be linked to radicals originating from cellulose [21].

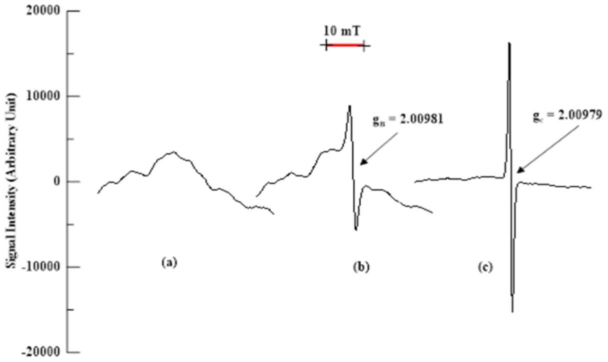

Figure 2. Spectra of unirradiated kernel (**a**), 10 kGy gamma-irradiated kernel (**b**), and hazelnut shell (**c**).

3.2. Microwave Power Saturation Behavior

Figure 3 illustrates how altering the microwave power impacts the peak-to-peak amplitude (HPP) of both the hazelnut kernel signal (S_b) and the shell signal (S_c). As depicted in the figure, the S_b demonstrates a linear growth until reaching a microwave power of 4 mW. Afterward, saturation begins, leading to a nonlinear rise in HPP that persists until the upper limit of the investigated range (160 mW).

For S_b, H_{PP} increases linearly up to 1.01 mW microwave power, beyond this value saturation in H_{PP} can be noticed easily till reaching the maximum value at microwave power of 25.0 mW where H_{PP} starts to decrease till the end of the studied range.

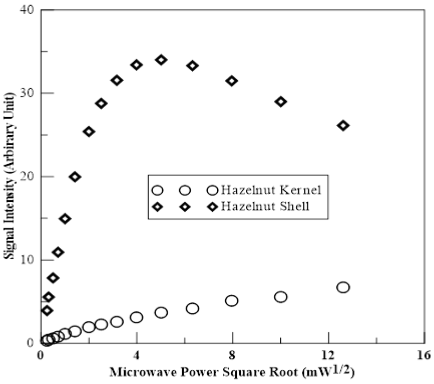

Figure 3. The correlation between the square root of microwave power ($mW^{1/2}$) and the peak-to-peak heights (HPP) for S_b (represented by circles) and S_c (represented by diamonds).

3.3. Effect of Modulation Amplitude

Figure 4 represents the impact of the change in modulation amplitude on the peak-to-peak amplitude (H_{PP}) of S_b and S_c. From this figure, H_{PP} increases as the modulation amplitude increases for both cases: kernels (represented by circles) and shells (represented by diamonds) the linear behavior cannot be guaranteed after the value of 0.2 mT modulation amplitude where saturation behavior dominates.

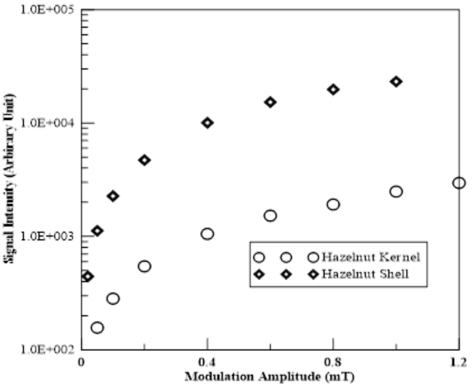

Figure 4. Effect of magnetic field modulation amplitude on peak-to-peak heights, H_{PP} (S_b) (Circles) and H_{PP} (S_c) (diamonds).

3.4. Time Dependence

The time dependence of H_{PP} for both kernel and shell signals was monitored closely over the first six hours after irradiation terminates in order to trace the dynamics of the radiation-induced radicals. During the first hour after irradiation, H_{PP} was stable within 2.4% for kernels and 0.80% for shells, while during the second hour it was stable within 2.7% and 1.6% and within 2.6% and 2.7% during the following four hours after irradiation for kernels and shells respectively, time dependence for radiation-induced radicals in hazelnut kernel and shell over the first six hours following the irradiation process is plotted in Figure 5.

For longer time periods, study of time dependence gives an idea about the stability of the radiation-induced radicals which enables detection and identification of the irradiated hazelnut. Figure 6 represents the change in H_{PP} for both of S_b and S_c over 38 days following irradiation, where the diamonds denote for hazelnut kernel and circles denote for shell. Dramatic decrease in H_{PP} for S_b and Sc to 72.9% and 76.7% of their original values was noticed after 9 days following irradiation, and to 46.9% and 51.3% after 23 days, while at the end of the study interval (after 38 days), H_{PP} decreased to 42.9% and 42.6% for S_b and S_c respectively.

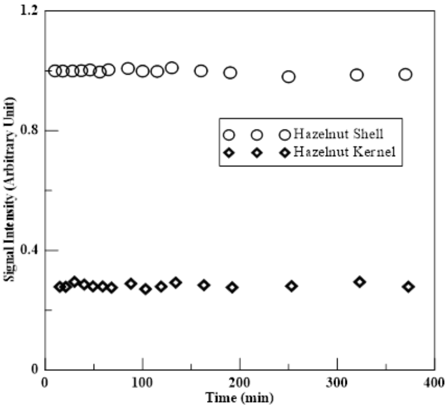

Figure 5. Short-term time dependence of peak-to-peak heights, H_{PP} (S_b) and H_{PP} (S_c) over the first six hours immediately after irradiation.

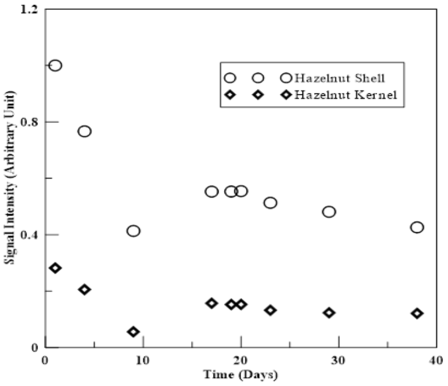

Figure 6. Long-term time dependence of peak-to-peak heights, H_{PP} (S_b) and H_{PP} (S_c) over the first 30 days following the day of irradiation.

3.5. Response to Different Radiation Doses

Figure 7 illustrates the response of hazelnut shells and kernels to varying radiation doses. Within the graph, H_{PP} values for both S_b and S_c were plotted against the radiation doses delivered to the hazelnuts over the range of 0.5 to 10 kGy. Evidently, as the radiation dose increases, both H_{PP} (S_b) and H_{PP} (S_c) exhibit an increasing behavior. Notably, the ratio between the response of H_{PP} (S_b) and H_{PP} (S_c) was noticed to fluctuate from 19.3% to 24.3% based on the dose level. This confirms the sensitivity of hazelnut shells to ionizing radiation compared to kernels

Response to ionizing radiation data was fitted using the power fit according to the equation: $\ln Y = B. \ln X + A$, where B and A are constants as follows: for kernels: A = 2.41 and B = 0.385, where values for shells are: A = 3.93 and B = 0.422.

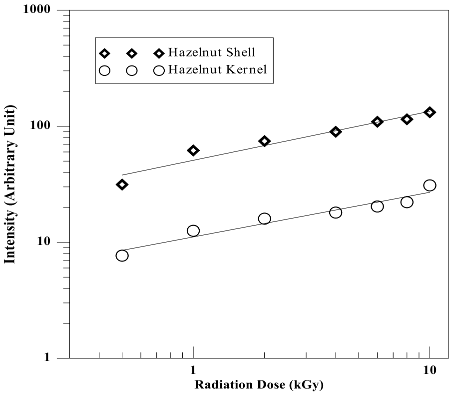

Figure 7. Response of peak-to-peak heights, H_{PP} (S_b) and H_{PP} (S_c) (arbitrary units) to different radiation doses over the range (0.5:10 kGy).

4. Conclusions

EPR is a powerful tool for the identification of irradiated hazelnut and also for the estimation of irradiation doses through samples of hazelnut shells and kernels. Hazelnut shells are much sensitive to radiation than kernels (four to five times) and can give more reliable results, however, kernels can be used for the estimation of irradiation doses in cases of the absence of hazelnut shells. Radiation-induced EPR signals (for kernel and shell) can persist for more than a month although the predictable decay, however, it is recommended to record the date of irradiation in order to perform necessary corrections and to perform the test within one month after the irradiation process. More irradiated food items are to be identified using EPR in the future.

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curation. A.M.: methodology. E.M.: conceptualization. All authors have read and agreed to the published version of the manuscript.

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