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Identification of Irradiated Cashew Nut by Electron Paramagnetic Resonance Spectroscopy

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Cashew nut samples were irradiated at γ-radiation doses of 0.25, 0.5, 0.75, and 1 kGy, the permissible dose range for insect disinfestation of food commodities. A weak and short-lived triplet (g = 2.004 and hfcc = 30 G) along with an anisotropic signal ($g_{\perp}$ = 2.0069 and $g_{||}$ = 2.000) were produced immediately after irradiation. These signals were assigned to that of cellulose and CO_2^- radicals. However, the irradiated samples showed a dose-dependent increase of the central line (g = 2.0045 ± 0.0002). The nature of the free radicals formed during conventional processing such as thermal treatment was investigated and showed an increase in intensity of the central line (g = 2.0045) similar to that of irradiation. Characteristics of the free radicals were studied by their relaxation and thermal behaviors. The present work explores the possibility to identify irradiated cashew nuts from nonirradiated ones by the thermal behaviors of the radicals beyond the period, when the characteristic electron paramagnetic resonance spectral lines of the cellulose free radicals have essentially disappeared. In addition, this study for the first time reports that relaxation behavior of the radicals could be a useful tool to distinguish between roasted and irradiated cashew nuts.

KEYWORDS: Food irradiation; detection of irradiated foods; EPR; cashew

INTRODUCTION

The cashew nut of the plant, *Anacardium occidentale*, is an important cash crop for many countries. It has high nutritive value; however, climatic conditions and variety govern quality (*1*). The kernel contains approximately 21% protein, 46% fat, and 25% carbohydrates. Cashew nut is an important export commodity for India, contributing about 7% to the national treasury. Insect infestation during storage is a major problem of cashew nut, resulting in economic losses. Irradiation is increasingly being recognized as an effective technology to reduce postharvest losses and improve quality. Cashew nuts, irradiated at 0.25 kGy and higher doses and stored under ambient conditions, showed no insect infestation during storage, while the control nonirradiated samples were spoiled due to infestation (*2*).

To facilitate trade in irradiated foods, regulatory authorities are interested in having a reliable method to detect irradiated foods and consequently check compliance with the labeling requirements. Methods to distinguish between irradiated and nonirradiated food stuffs are useful to both enforcement agencies and consumers to increase confidence in radiation processing technology.

Several detection methods have been developed for the identification of irradiated foods (*3*). Among them, electron paramagnetic resonance (EPR) spectroscopy is a leading technique. For use in detection, the radiation-induced EPR signals in food must fulfill several requirements; that is, they must be stable or fairly stable during the usual storage period of the foodstuff and must be clearly distinguishable from the background signals of the nonirradiated sample, even after a long storage period (*4*). Three European standards for the detection of irradiated food by EPR spectroscopy have been released by the European Committee of Normalization (CEN) and adopted by Codex Alimenterius Commission as Codex Standards. These pertain to food containing bone (*5*), crystalline sugar (*6*), and cellulose (*7*). This last standard has been validated for pistachio shells, paprika powder, and fresh strawberries (*8*−*11*). EPR is a user friendly technique, as the measurement is easy and the sample under test does not require any preparation, and there is increasing interest in extending the EPR methodology to other types of food containing cellulose. The main problem lies in the instability of the relatively weak satellite lines of cellulose. In some cases, the satellite lines are not detectable immediately after irradiation. Recently, to extend the applicabil-

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ity of EPR for the identification of irradiated food, a new approach, based on thermal treatment and EPR saturation, has been used when the satellite lines due to cellulose have presumably disappeared due to long periods of time elapsed after treatment (*12, 13*).

In the present work, a detailed study of the radical species produced by γ-irradiation of cashew has been carried out. In addition, the EPR saturation and thermal behaviors to distinguish between treated and untreated samples by EPR spectroscopy have been examined.

EXPERIMENTAL PROCEDURES

Materials. Cashew nuts were procured from a local market. The standard 2,2-diphenyl-1-picrylhydrazyl (DPPH) with $g = 2.0032$ was purchased from Sigma Chem. Co. (United States).

Irradiation Conditions. Irradiation was carried out at ambient temperature (27 ± 2 °C) using a cobalt-60 irradiator (GC-5000, BRIT, Mumbai, dose rate of 6.2 kGy/h) at BARC, Mumbai. The doses were applied in the range of 0.25−1 kGy, recommended for insect disinfestation. Dosimetry was performed using an aqueous Fricke dosimeter (*14*). The irradiated, roasted, and nonirradiated cashew nut samples were stored inside EPR quartz tubes in the normal laboratory conditions at ambient temperature (27 ± 2 °C) until further use.

EPR Spectroscopy. Cashew nut kernels were cut into fine pieces and transferred to 2 mm quartz capillary tubes and packed with gentle tapping to a length of 25.4 mm (active length), and the weight of the sample was determined. The results for the signal intensity of samples were normalized to the packing weight. EPR measurements were performed using a Bruker EMX spectrometer (Bruker, Gemany). All of the spectra were recorded at ambient temperature of the EPR laboratory (27 °C).

Operating conditions of the EPR spectrometer were as follows: center field, 3480 G; scan range, 200 G; microwave power, 0.253 mW; microwave frequency, 9.66 GHz; modulation frequency, 100 kHz; receiver gain, 4×10^4; and time constant, 20.48 s. The position of the irradiation-induced EPR signal was compared with that of the standard DPPH with $g = 2.0032$ (Sigma Chem. Co.).

Progressive Saturation and Thermal Behavior of Radicals. To determine the electron relaxation behavior of radicals in cashew nut samples, the microwave field strength was varied between 0.06 and 50 mW to obtain progressive saturation behavior (PSB). The field modulation was operated at 100 kHz. All of the EPR measurements were done at ambient temperature. The spin concentration was determined using DPPH as a standard sample.

In situ heat treatment from room temperature to 187 °C in a step of 20 °C was conducted using nitrogen gas for heating the samples within the EPR spectrometer using BVT-3000 accessory of Bruker (Germany). To study the induced radicals by thermolysis, heat treatment before and after irradiation of the samples was carried out at 100 °C for 1 h and roasting at 180 °C using a laboratory oven. Three replications were made for the evaluation of each sample.

RESULTS AND DISCUSSION

Effect of γ-Irradiation. Figure 1, spectrum **a**, shows the EPR signal of cashew nut samples before irradiation exhibiting a weak and broad singlet characterized by $g = 2.0056 \pm 0.0004$ centered around 3475 G. This g value obtained compares well with those reported in literature (*13, 15*). The origin of these free radicals responsible for the EPR signal is not clear. Several reports have suggested these free radicals to be those of semiquinones produced by the oxidation of plant polyphenolics (*16*) or lignin (*17, 18*). To characterize the natural signal, EPR spectra of irradiated (1 kGy) pure quinone (hydroquinone) were recorded under a similar experimental setup. A weak singlet was observed with $g = 2.0032$ and was different from the signal observed in nonirradiated sample. This suggests that, in cashew nut, where the lipid content is more than 40%, the origin of the singlet could possibly be due to the oxidation of fatty acids (*19*).

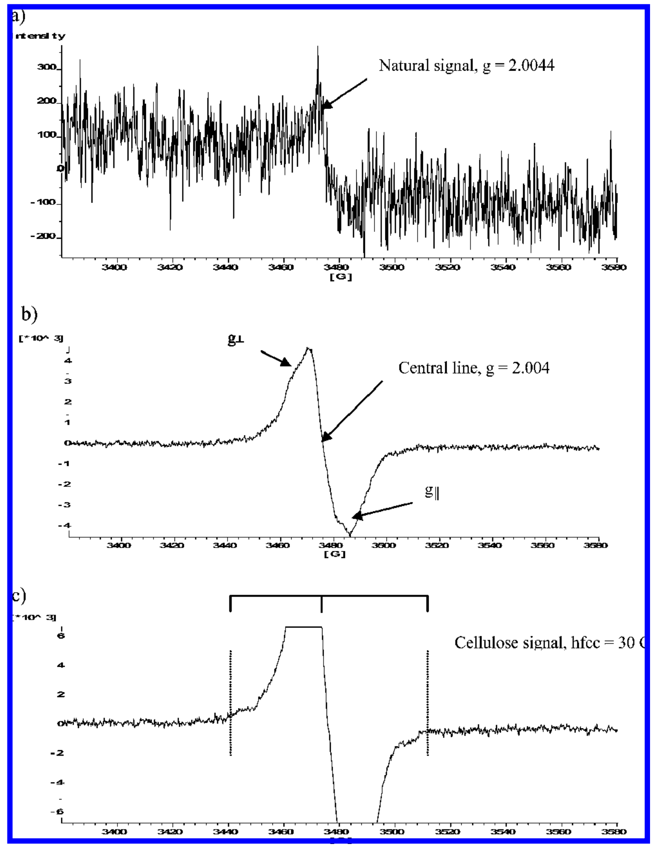

Figure 1. Ambient EPR spectra of cashew nut sample (**a**) before irradiation, (**b**) 1 day after irradiation (1 kGy), and (**c**) irradiated (1 kGy) and magnified.

Figure 1, spectrum **b**, shows a complex spectrum immediately after irradiation (1 kGy) of cashew samples with an increase in signal intensity of the existing weak singlet by a factor of 11. Similar observations were also reported by Raffi et al. (*15*), where the intense signal was noticed in the spectrum of irradiated spices. Irradiation was explained to be responsible for the relatively high intensity increase. The exposure to γ-irradiation leads to a change in the cashew matrix, producing two new types of paramagnetic species. One was identified as a very weak triplet signal probably because of crystalline cellulose (*20, 21*) superimposed on the natural singlet with a hyperfine coupling constant (hfcc) of 30 G (**Figure 1**, spectrum **c**). This signal is considered to be unambiguous evidence of the radiation treatment of sample under investigation and recommended for the detection of irradiated foods in EN 1787 standard (*7*). The same has been validated for pistachio shells by Raffi et al. (*9*). Another short-lived paramagnetic species with an axially symmetric spectrum (**Figure 1**, spectrum **b**) was characterized by an anisotropic g tensor ($g_\perp = 2.0069$ and $g_\parallel = 2.0000$). This signal could possibly be of CO_2^- radical formed during the breakdown of fatty acid. DPPH with $g = 2.0032$ was used as a reference to calculate the g values of the radicals. The g values obtained compared well with those reported in the literature (*22, 23*). This component was not particularly stable and, after 2 days, had decreased in intensity to a level that was similar to that of nonirradiated specimen. The instability in signal intensity means detection of fat component by EPR spectroscopy is limited after irradiation.

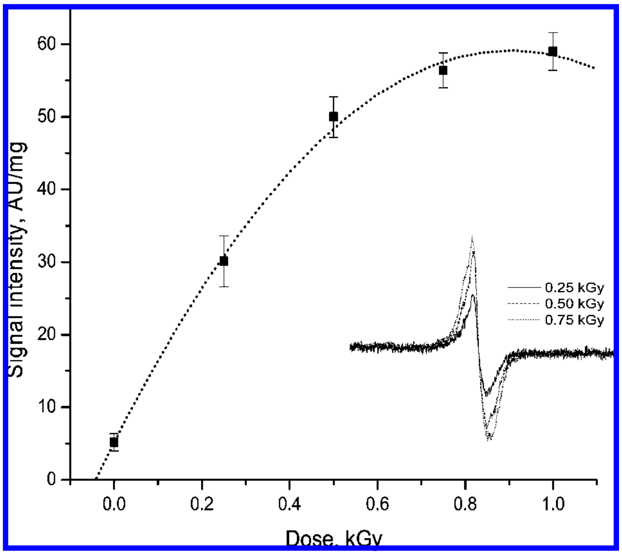

Figure 2. Response of the radiation-induced signal intensity of the central line ($g = 2.0044$) with increasing radiation dose.

The effects of increasing radiation dose from 0.25 to 1.0 kGy on the spectra of cashew samples were studied. The radiation-induced signal due to CO_2^- radical started developing at a minimum dose of 0.25 kGy, whereas the signal of cellulose radical was identified at 1.0 kGy dose. However, the relative intensity of the central line was observed to be significantly related to the radiation dose as shown in **Figure 2**. The measurement was performed 3 days after irradiation. The dotted line represents a second order polynomial curve $D = aD^2 + bD + c$ with $a = -66.19$, $b = 119.56$, and $c = 5.15$ and each point representing the mean value of three samples.

Effect of Thermal Treatment. Radiation-induced radicals formed from fat components of the cashew matrix are highly unstable. EPR spectra of irradiated and nonirradiated samples after storage showed similar patterns except for a significant enhancement in the natural signal intensity after irradiation. To validate a method for identifying irradiation, free radicals produced by other processing techniques such as heating (thermolysis) must be distinguished from those produced by irradiation. **Figure 3**, spectra **a**−**c** show the EPR spectra of cashew nut samples subjected to various thermal treatments before and immediately after heating. Only a weak singlet (**Figure 3**, spectrum **b**) similar to the natural signal was observed after thermal treatment of the whole sample at 100 °C for 1 h. Radicals originated due to thermolysis characterized by $g = 2.0045 \pm 0.0005$, centered around 3476 G. No satellite lines like cellulose and CO_2^- radicals were observed. The samples subjected to roasting at 180 °C showed increased signal intensity (**Figure 3**, spectrum **c**) similar to that of irradiated samples, making detection of radiation treatment of cashew difficult.

Characterizations of Nonirradiated, Irradiated, and Heat-Treated Cashew Nuts by EPR Saturation and Thermal Behavior. The applicability of the EPR analysis to detect irradiated food is strongly limited by the lifetime of the radiation-induced free radicals such as cellulose radicals. This is the case with cashew nut under investigation where the procedure based on EPR cellulose signal (*7*) cannot be used beyond 1 day of radiation treatment. Therefore, alternative methods proposed in the European Standard EN 1787 (*7*) and based on EPR are desirable. Characterization of the free radicals, naturally present, induced by radiolysis or by thermolysis was essential for the investigation to identify different techniques of processing. To determine the electron relaxation behavior of radicals in the cashew, we varied the microwave field strength from 0.063 to 50 mW to obtain PSB. The effect of saturation is manifested by a continuous nonlinear increase of EPR signal intensity with $P_{MW}^{1/2}$, reaching a maximum followed by a decrease with the simultaneous increase of EPR line width. **Figure 4a** shows the PSB of the central line of irradiated (1 kGy), roasted (180 °C), and nonirradiated samples 1 day after radiation and heat treatments. A comparatively faster saturation at microwave power around 6 mW followed by a decrease in signal intensity by monotonic fashion was observed for the radicals formed after roasting. This saturation behavior revealed the characteristics of organic radicals with large relaxation time. Improvement of the EPR detection of irradiated dry food using microwave saturation and thermal treatment has been proposed by Yordanov et al. (*12*). In this method, curves of saturation of nonirradiated and irradiated plants vs $P_{MW}^{1/2}$ were studied, and nonirradiated samples showed saturation at microwave power higher than 15 mW, whereas an irradiated sample exhibited early saturation at microwave power of around 8 mW. In the present study, no significant difference of saturation behaviors of nonirradiated and irradiated cashew nut was observed. The central line of the spectra for both nonirradiated and irradiated samples exhibited similar saturation at microwave power around 20 mW, revealing shorter relaxation time. As depicted in **Figure 4b**, the relaxation behaviors of nonirradiated, irradiated, and roasted samples, even after a storage period of 45 days, were almost similar to that of behavior noticed immediately after irradiation and heat treatment. Therefore, EPR analysis by the saturation behavior of the signals could not be used to distinguish irradiated cashew sample. However, faster EPR saturation of thermally induced radicals in comparison with the radiation-induced radicals could be a tool to distinguish between irradiated and roasted cashew.

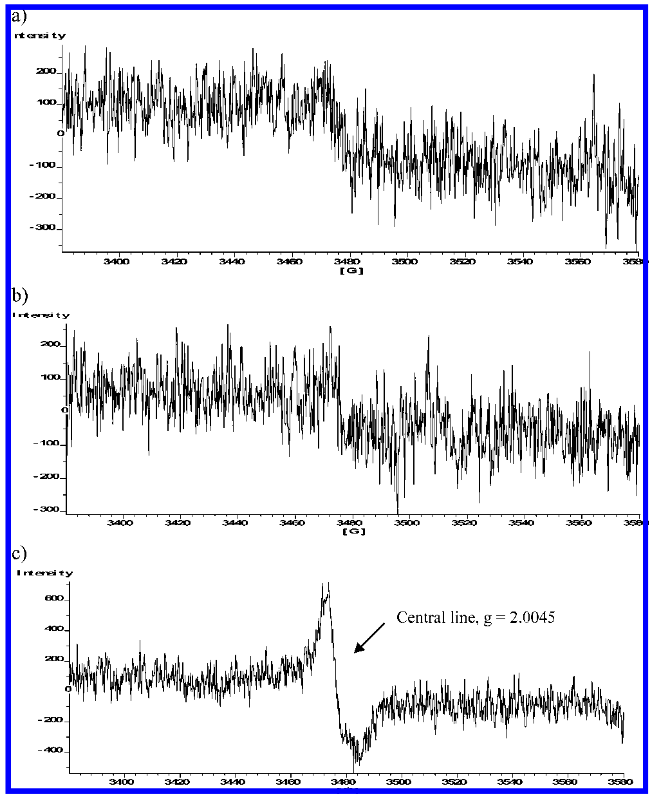

Figure 3. EPR spectra after 1 day of various thermal treatments (**a**) without thermal treatment, (**b**) subjected to 100 °C for 1 h, and (**c**) roasted at 180 °C.

To identify the irradiated cashew sample from natural and roasted samples, the thermal behavior of the EPR signal was

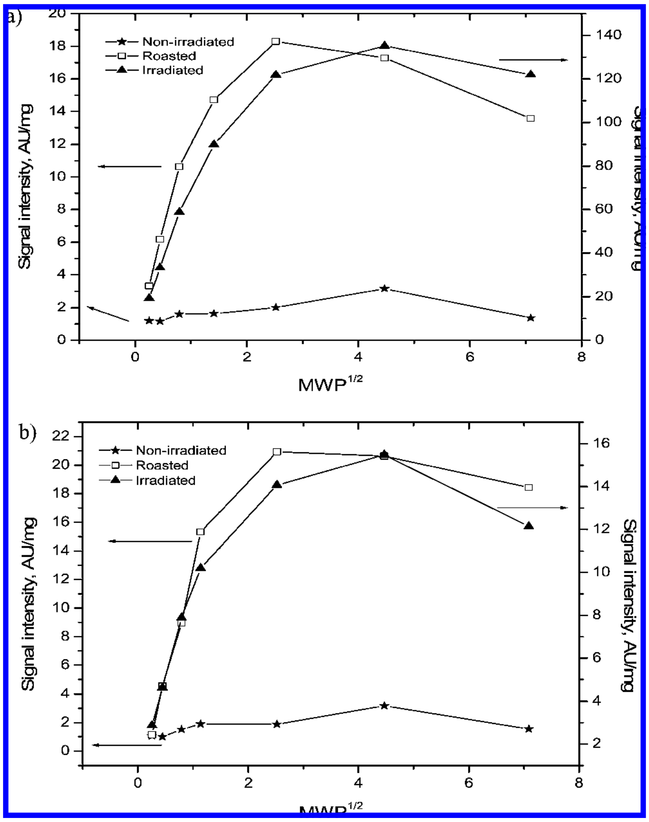

Figure 4. Relaxation behavior of main line of nonirradiated, irradiated (1 kGy), and roasted cashew (**a**) 1 day after irradiation and (**b**) after 45 days of storage.

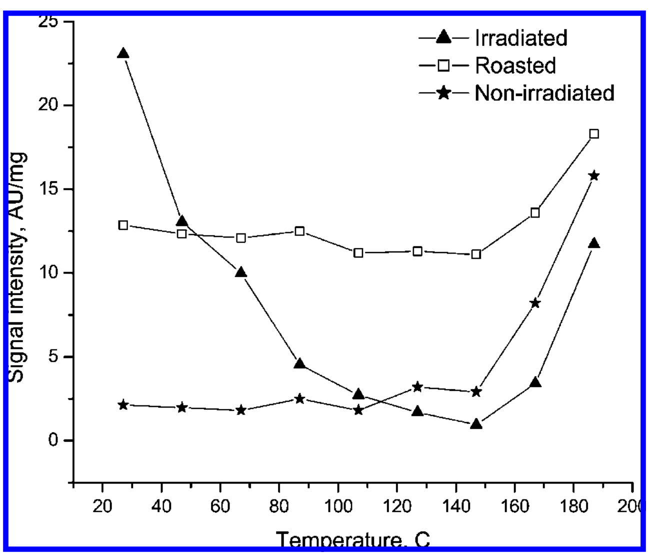

Figure 5. Thermal behavior of the central line of EPR spectrum for nonirradiated, irradiated (1 kGy), and roasted (180 °C) cashew nut samples.

investigated. Nonirradiated, irradiated, and roasted samples were subjected to in situ heating from 27 to 187 °C. As depicted in **Figure 5**, with the increase of temperature from 27 to 97 °C, signal intensities of the central lines of nonirradiated and roasted samples were observed to be almost unchanged, but that of irradiated samples showed a fast fall of about 50%. However, a temperature increase beyond 152 °C lead to an increase in signal intensities in all of the cases. This could probably be due to the decomposition of the samples and the formation of new thermally induced radicals. Polovka et al. (*24*) have recently investigated the thermal behavior of irradiated and nonirradiated spices. A decline of EPR signal of irradiated allspice sample was reported. Yordanov and Gancheva (*13*) proposed that EPR analysis of the central peak of spices that had undergone thermal treatment before and a relatively long time after irradiation could be a tool for detection of irradiation. To establish this method, thermal behaviors of the samples were investigated after a storage period of 6 weeks. Nonirradiated, roasted, and irradiated samples were subjected to thermal treatment of 100 °C for 1 h. Irradiated samples showed 39% reduction in signal intensity, whereas nonirradiated and roasted samples did not exhibit any significant change in signal intensity. Our results are well in agreement with the proposal of Yordanov and Gancheva (*13*). As the EPR signals of cellulose required by the European Standards were not visible in cashew samples after a long period of storage, the application of Protocol EN 1787 (*7*) was impossible. Therefore, the thermal behavior of the γ-induced EPR signal represented a valuable tool for the assessment of previous radiation treatment.

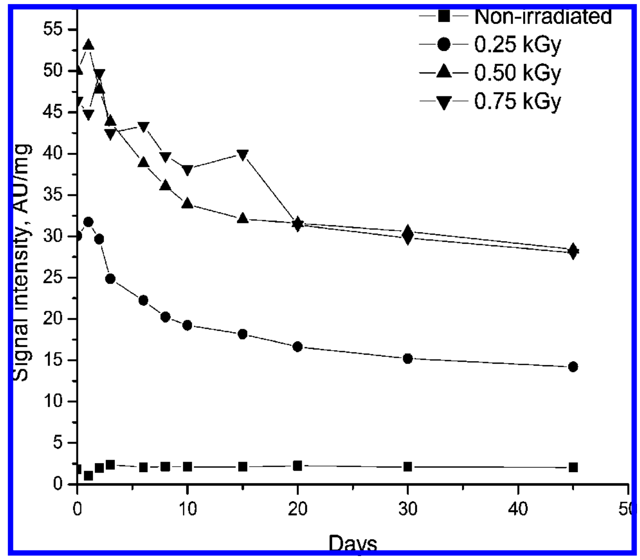

Figure 6. Kinetics of the central line of the EPR signal of nonirradiated and irradiated cashew nut samples.

Kinetic Study. The fading kinetics of the radiation-induced radicals give information on the time interval after which identification of the irradiated samples is possible. Marked decreases in the concentration of free radicals in irradiated spices with time have been reported by many authors (*13, 15, 25*). Different factors, including humidity, temperature, light intensity, exposure to air, and structure of the food matrix influence the behavior of the induced radicals during storage of the sample before and after irradiation and, thereby, restrict the time interval after irradiation during which detection is possible. To avoid these variations, all of the samples were kept inside the EPR measurement tube in normal laboratory conditions. Kinetics of nonirradiated, irradiated, and roasted samples were monitored up to 6 weeks after radiation treatment. Both of the radiation-induced paramagnetic species identified as cellulose radicals and CO_2^- radical were observed to be stable up to 24−40 h after irradiation in the matrix of cashew nut. Therefore, application of EN 1787 Standard (*7*) was not possible as a method of detection. However, increased intensity of the central line of the irradiated samples was observed even after several days of irradiation with a clear distinction with respect to nonirradiated samples (**Figure 6**).

In conclusion, investigation of free radicals in γ-irradiated cashew nuts by EPR spectroscopy showed a proportional increase in free radical concentration with an increase of irradiation dose. A weak singlet was found in the commercially available cashew nuts. However, two distinct but short-lived signals were identified as cellulose and CO_2^- radicals in irradiated sample. By increasing the microwave power, the line shape of EPR spectra altered, and from the saturation curves, it was possible to identify the irradiated and roasted samples. However, radicals present naturally were not distinguishable from the irradiated signal by the respective relaxation behavior. The thermal behavior of the radicals after storage clearly showed the distinction between naturally present and radiation-induced radicals. Identification of radiation treatment after storage of the sample under investigation was not possible by the application of protocol EN 1787 (*7*). However, irradiated cashew nut can be distinguished from nonirradiated samples after a long period of storage by the thermal behavior of the EPR signals. In addition, this report for the first time proposes a method to distinguish between irradiated and roasted sample by studying the relaxation characteristics of the induced radicals.

LITERATURE CITED

(1) Ohler, J. G. *Cashew*; Department of Agricultural Research, Royal Tropical Institute: Amsterdam, 1979.
(2) Bhattacharjee, P.; Singhal, R. S.; Gholap, A. S.; Variyar, P. S.; Bongirwar, D. R. Compositional profiles of γ-irradiated cashew nuts. *Food Chem.* **2003**, *80* (2), 151–157.
(3) Chung, H. W.; Delincee, H.; Han, S.; Hong, J.; Kim, H.; Byun, M.; Know, J. Trials to identify irradiated chestnut (*Castanea bungena*) with different analytical techniques. *Radiat. Phys. Chem.* **2004**, *71* (1−2), 181–184.
(4) Raffi, J. J.; Agnel, J. L.; Buscarlet, L. A.; Martin, C. C. Electron spin resonance identification of irradiated strawberries. *J. Chem. Soc. Faraday* **1988**, *84* (10), 3359–3362.
(5) Detection of Irradiated Food Containing Bone: Analysis by Electron Paramagnetic Resonance; Protocol EN 1786; European Committee for Standardization: Brussels, 1997.
(6) Detection of Irradiated Food Containing Crystalline Sugar: Analysis by EPR; Protocol EN 13708; European Committee for Standardization: Brussels, 2001.
(7) Detection of Irradiated Food Containing Cellulose: Analysis by EPR; Protocol EN 1787; European Committee for Standardization: Brussels, 2000.
(8) Desrosiers, M. F. Current status of EPR method to detect irradiated food. *Appl. Radiat. Isot.* **1996**, *47*, 1621–1628.
(9) Raffi, J.; Stevenson, M. H.; Kent, M.; Thiery, J. M.; Belliardo, J. J. European intercomparison on electron spin resonance identification of irradiated food stuffs. *Int. J. Food Sci. Technol.* **1992**, *27*, 111–124.
(10) Schreiber, G. A.; Helle, N.; Schulzki, G. Intercomparisons to evaluate the suitability of gas chromatographic, electron spin resonance spectrometric and thermoluminescence methods to detect irradiated foods in routine control. *Radiat. Phys. Chem.* **1993**, *43*, 391–396.
(11) Schreiber, G. A.; Helle, N.; Schulzki, G. Interlaboratory tests to identify irradiation treatment of various food stuffs via gas chromatographic detection of hydrocarbons, ESR spectroscopy and TL analysis. *Detection Methods for Irradiated Foods—Current Status*; Royal Society of Chemistry: Cambridge, United Kingdom, 1996; pp 98−107.
(12) Yordanov, N. D.; Aleksieva, K.; Mansour, I. Improvement of the EPR detection of irradiated dry plants using microwave saturation and thermal treatment. *Radiat. Phys. Chem.* **2005**, *73*, 55–60.
(13) Yordanov, N. D.; Gancheva, V. A new approach for the extension of the identification period of irradiated cellulose containing foodstuffs by EPR spectroscopy. *Appl. Radiat. Isot.* **2000**, *52*, 195–198.
(14) ASTM Standard, E 1026. Standard Practice for Using Fricke Reference Standard Dosimetry System.
(15) Raffi, J.; Yordanov, N. D.; Chabane, S.; Douifi, L.; Gancheva, V.; Ivanova, S. Detection of irradiated aromatic herbs and spices by electron paramagnetic resonance and thermoluminescence. *Spectrochim. Acta* **2000**, *56A*, 409–416.
(16) Scewartz, H. M., Bolton, J. R., Brog, D. C., Eds. *Biological Applications of Electron Spin Resonance*; Wiley: New York, 1972.
(17) Maloney, D. R.; Tabner, B. J.; Tabner, V. A. An electron spin resonance study of gamma-irradiated fruits. *Radiat. Phys. Chem.* **1992**, *39*, 309–314.
(18) Tabner, B. J.; Tabner, V. A. Electron spin resonance spectra of gamma-irradiated citrus fruits skin, skin components and stalks. *Int. J. Food Sci. Technol.* **1994**, *29*, 143–152.
(19) Ikeya, M.; Baffa, O. F.; Mascarenhas, S. Quality assessment of coffee beans with ESR and gamma- irradiation. *Appl. Radiat. Isot.* **1989**, *40*, 1219–1222.
(20) Raffi, J.; Agnel, J. P. L. Electron spin resonanace identification of irradiated fruits. *Radiat. Phys. Chem.* **1989**, *34*, 891–894.
(21) Bortolin, E.; Butos Griffin, E.; Cruz-Zaragoza, E.; De Coste, V.; Onori, S. Electron paramagnetic resonance detection of Mexican irradiated spices. *Int. J. Food Sci. Technol.* **2006**, *41*, 375–382.
(22) Geoffroy, M.; Tochon-Danguy, H. J. ESR identification of radiation damage in synthetic apatites: a study of the carbon-13 hyperfine coupling. *Calcif. Tissue Int.* **1982**, *34*, S99–S102.
(23) Marchioni, E.; Horvatovich, P.; Charon, H.; Kuntz, F. Detection of irradiated ingredients included in low quantity in non-irradiated food matrix. 1. Extraction and ESR analysis of bones from mechanically recovered poultry meat. *J. Agric. Food Chem.* **2005**, *53*, 3769–3773.
(24) Polovka, M.; Brezova, V.; Simko, P. EPR spectroscopy: A tool to characterize gamma irradiated foods. *J. Food Nutr. Res.* **2007**, *46*, 75–83.
(25) Delincee, H. Detection of food treated with ionizing radiation. *Trends Food Sci. Technol.* **1998**, *9*, 73–82.

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