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Analytical Methods

An improved method to identify irradiated rice by EPR spectroscopy and thermoluminescence measurements

Bhaskar Sanyal *, S.P. Chawla, Arun Sharma

Food Technology Division, Bhabha Atomic Research Centre, FIPLY, Trombay, Mumbai, Maharashtra 400 085, India

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ABSTRACT

Minimal change in irradiated foods with low dose treatment makes the identification process a difficult task. Two independent physical methods, electron paramagnetic resonance (EPR) spectroscopy and thermoluminescence (TL) detection were employed for detection of irradiation treatment on Basmati rice. EPR investigation of 0.5–2.0 kGy irradiated rice samples showed a short lived, asymmetric, dose dependent spectrum ($g = 2.005$), characterised by the radicals of irradiated starch. However, this signal disappears with time. The present work explores the possibility to identify irradiated rice by the relaxation characteristics and thermal behaviour of the radicals. This study reports for the first time that the different microwave saturation behaviours of the signal ($g = 2.004$) in irradiated and non-irradiated rice samples provide an important clue to identify radiation treatment beyond the period when the radiation specific EPR spectral lines have disappeared. TL investigation involving scanning electron microscopy/energy dispersive X-ray analysis (SEM/EDX) of the poly-minerals isolated from the rice samples allowed to discriminate clearly between irradiated and non-irradiated samples even after a prolonged period of storage.

1. Introduction

Rice is an important cash crop and staple food for many countries. It has high nutritive value and the quality is governed by variety and climatic conditions. The eating quality of rice has long been ascribed to its starch. Starch accounts for 95% of the dry matter in milled rice grain (Martin & Fitzgerald, 2002). Insect infestation during storage is a major problem of rice, resulting in economic losses. Irradiation is increasingly being recognised as an effective technology to reduce post-harvest losses and improve quality. Irradiated (0.25–1.0 kGy) Basmati rice, a high value fragrant rice of India, did not exhibit insect infestation during a storage period of 6 months, at room temperature that varied between 27 °C and 33 °C and relative humidity between 59% and 87%, while the control (non-irradiated) samples were spoiled due to infestation. During sensorial evaluation, no significant difference was found between the acceptability of irradiated and non-irradiated (control) rice (Sudha Rao, Gholap, Adhikari, & Madhusudanan, 2000). These results are significant in view of the high export potential of Basmati rice and the losses attributed to infestation.

To facilitate trade in irradiated foods, regulatory authorities need a reliable method to detect irradiated foods and consequently check compliance with the labelling requirements. In view of the growing interest in irradiation technology of the food industry, development of reliable methods to distinguish between irradiated and non-irradiated food stuffs is essential for the benefit of enforcement agencies as well as consumers to increase confidence in radiation processing technology.

Several detection methods have been developed for identification of irradiated foods (Chung et al., 2004). Among them, EPR spectroscopy and thermoluminescence detection are the two leading techniques. For use in detection, radiation induced EPR signals in food must fulfil several requirements, i.e. 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 non-irradiated sample (Raffi, Agnel, Buscarlet, & Martin, 1988). Three European standards for detection of irradiated food by EPR spectroscopy have been released by European Committee of Normalisation (CEN) and adopted by Codex Alimenterius Commission as Codex Standards. These pertain to food containing bone (EN 1786, 1997), cellulose (EN 1787, 2000) and crystalline sugar (EN 13708, 2001). This last standard has been validated for irradiated skin of raisins and figures containing considerable amount of polysaccharides in the form of sugar. EPR is a user friendly technique, as the measurement is easy and the sample under test does not require any preparation. There is an increasing interest in extending the EPR methodology to various types of food. The main problem lies in the instability of the relatively weak radiation specific signals. Recently, in order to extend the applicability of EPR for identification of irradiated food, a new approach, based on thermal treatment and EPR saturation have been used when the radiation

* Corresponding author. Tel.: +91 22 25592533; fax: +91 22 25505151/25519613.
E-mail addresses: bhaskar_sanyal@rediffmail.com, sanyal@barc.gov.in, bhaskar_barc@yahoo.com (B. Sanyal).

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induced signals have disappeared due to long period of time elapsed after treatment (Yordanov, Aleksieva, & Mansour, 2005; Yordanov & Gancheva, 2000).

A negligible change in food commodities after radiation treatment makes the identification of irradiated food an extremely challenging task and the statistical safety margin is rather small for a reliable verdict to be given. Therefore, one method alone is not reliable enough for the detection of a treatment by ionizing radiation. In view of this, in present work, two independent physical methods were employed. Such a combined approach may be suitable for overcoming certain limitations with detection of irradiated foods. Thermoluminescence (TL) is a radiation specific phenomenon that arises due to energy stored by trapped charge carriers following irradiation (Sanderson, Carmichael, Spencer, & Naylor, 1996). TL has been tested for detection of spices and herbs and was adopted as a standard method for detecting irradiated foods from which silicate minerals can be isolated (EN 1788, 2001).

In this present work a detailed study of the radical species produced by gamma irradiation of rice has been carried out by EPR saturation and thermal behaviour to distinguish between treated and non-treated samples. In addition, characterisation of the extracted minerals from the rice sample and the possibility of TL technique to identify irradiated and non-irradiated samples have been examined.

2. Experimental

2.1. Materials

Rice (Basmati) samples were procured from a local market. The standard 2,2,diphenyl-1-picrylhydrazyl (DPPH) with $g = 2.0032$ was purchased from Sigma Chem. Co. USA. Pure starch was procured from Himedia, India. Sodium polytungstate ($Na_6W_{12}O_{39} * H_2O$) was purchased from Fluka, Germany to prepare a high density solution.

2.2. 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 0.5–2 kGy in order to cover the recommended doses for insect disinfestations. Dosimetry was performed using aqueous Fricke dosimeter (ASTM Standard, E 1026).

2.3. EPR spectroscopy

Rice samples were ground into fine granules 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 normalised to the packing weight. EPR measurements were performed using Bruker EMX spectrometer (Bruker, Germany). All the spectra were recorded at the ambient temperature of EPR laboratory (27 °C).

Operating conditions of the EPR spectrometer were as follows: Centre 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 2,2,diphenyl-1-picrylhydrazyl (DPPH) with $g = 2.0032$ (Sigma Chem. Co. USA).

The irradiated and non-irradiated rice samples were stored inside EPR quartz tube in the normal laboratory conditions at ambient temperature (27 ± 2 °C) until further use.

2.4. Thermal and progressive saturation behaviour of radicals

In situ heat treatment from room temperature to 250 °C in a step of 25 °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 using a laboratory oven. Four replications were made for the evaluation of each sample.

In order to determine the electron relaxation behaviour of radicals in rice samples, the microwave field strength was varied between 0.06 and 50 mW to obtain progressive saturation behaviour (PSB). Field modulation was operated at 100 kHz. All the EPR measurements were carried out at ambient temperature. Spin concentration was determined using DPPH (1,1-diphenyl-2-picrylhydrazyl) as a standard sample.

2.5. Thermoluminescence analysis

For separation of minerals and organic materials from the rice sample, European Standards EN 1788, 2001 was followed. The important procedures involved in sample preparation for TL analysis were as follows.

The solution of sodium polytungstate ($Na_6W_{12}O_{39} * H_2O$) was added to double distilled water to prepare a high density solution (2 g/ml). The samples were subjected to ultrasonication in a bath sonicator for 5 min followed by centrifugation at 1000*g* for 2 min. The organic material floating on the top of the polytungstate solution was removed. The bottom layer was washed three times with deionised water. After total removal of water, 2 ml of acetone was added. The minerals in acetone were transferred to clean and weighed aluminium discs (diameter 9.0 mm; thickness 0.4 mm) with the help of Pasteur pipette. The discs containing minerals were stored overnight at 50 °C.

The discs containing minerals from control and irradiated samples were weighed to determine the quantity of minerals deposited. Thermoluminescence analysis was carried using TL 1009I Reader (Nucleonix Systems, India). Nitrogen was flushed in the heating chamber to reduce spurious TL arising due to the presence of oxygen. The initial temperature was 40 °C, which was increased to 300 °C by linear heating at a rate of 5 °C/s. After measurement of glow 1, the discs with the deposited mineral were irradiated with a normalisation radiation dose of 1 kGy followed by TL measurement of glow 2 with the similar instrumental settings as described above. The samples were separated and analysed in triplicate under similar laboratory and instrumental conditions. The irradiated and non-irradiated rice samples were stored in dark in the normal laboratory conditions at ambient temperature (27 ± 2 °C) until further use.

Scanning electron microscopy and energy dispersive X-ray spectrometer analysis (SEM/EDX) were carried out to determine the poly mineral composition of the isolated minerals from rice samples. The electron microscopic analysis was done with TESCAN, Czechoslovakia and EDX, INCA x – sisht, Oxford, UK with secondary electron detector.

3. Results and discussion

3.1. Effects of gamma irradiation

Fig. 1a, shows the EPR signal of non-irradiated rice samples exhibiting a weak singlet characterised by $g = 2.0049 \pm 0.0004$ and $\Delta B_{pp} = 13$ G, centered around 3461 G. DPPH with $g = 2.0032$ was used as a reference to calculate the *g-values* of the radicals. Similar singlet EPR line has been reported for other food

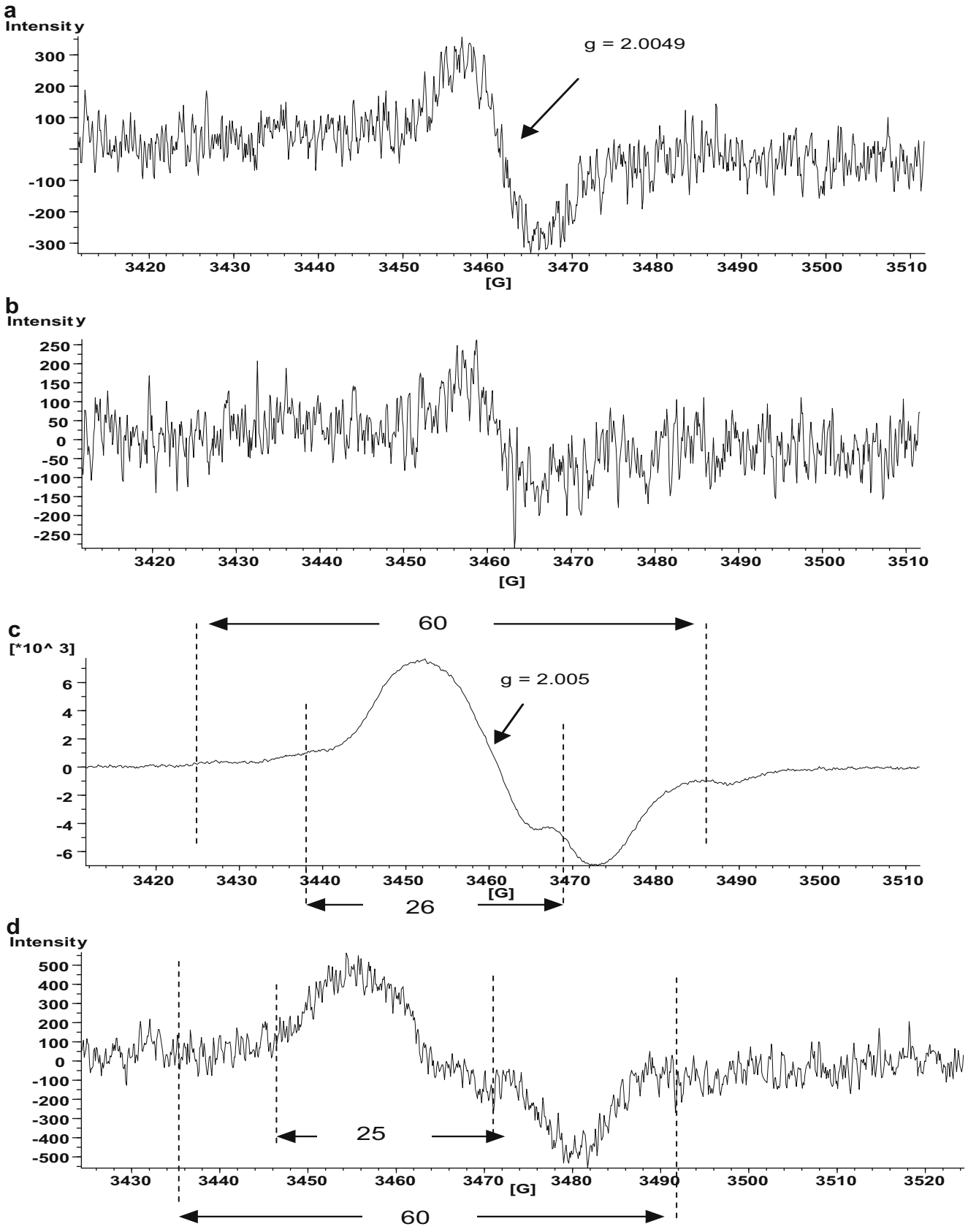

Fig. 1. EPR spectra of (a) non-irradiated (control), (b) heat treated at 100 °C, 1 h, (c) irradiated (1 kGy) rice samples and (d) EPR spectrum of irradiated pure starch.

commodities such as fresh strawberry (Desrosiers & McLaughlin, 1989; Dodd, Swallow, & Ley, 1985), grapes (Goodman, McPhail, & Duthie, 1989) and black pepper (Franco et al., 2004). The *g-value* obtained for non-irradiated rice sample compares well with those

reported in literature (Marchioni, Horvatovich, Charon, & Kuntz, 2005; Yordanov & Pachova, 2006). 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 semi-quinones produced by the oxidation of plant polyphenolics (Scewartz, Bolton, & Brog, 1972) or lignin (Maloney, Tabner, & Tabner, 1992; Tabner & Tabner, 1994).

In order 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. Fig. 1b shows the EPR signal of the rice sample subjected to thermal treatment at 100 °C for 1 h. The heat energy did not induce any specific change in the EPR spectrum. However, the intensity of the singlet (g = 2.0049) was observed to reduce by about 20%.

Fig. 1c shows complex spectrum immediately after irradiation (1 kGy) of rice samples with an increase in signal intensity of the existing weak singlet (g = 2.005). Similar observations were also reported by Raffi et al. (1988), where the intense signal was noticed in the spectrum of irradiated spices. Irradiation was explained to be responsible for the relatively high intensity increase. As depicted in spectrum c, the exposure to gamma irradiation leads to change in rice matrix, producing two new types of paramagnetic species. One pair of intense satellite lines at a distance of 60 G from each other and the other less intense pair of lines situated at a distance of 26 G from each other. The starch accounts for 95% of the dry matter in milled rice grain (Martin & Fitzgerald, 2002). In order to characterise this radiation specific signal, EPR spectrum of pure starch sample irradiated with 1 kGy dose of gamma radiation was

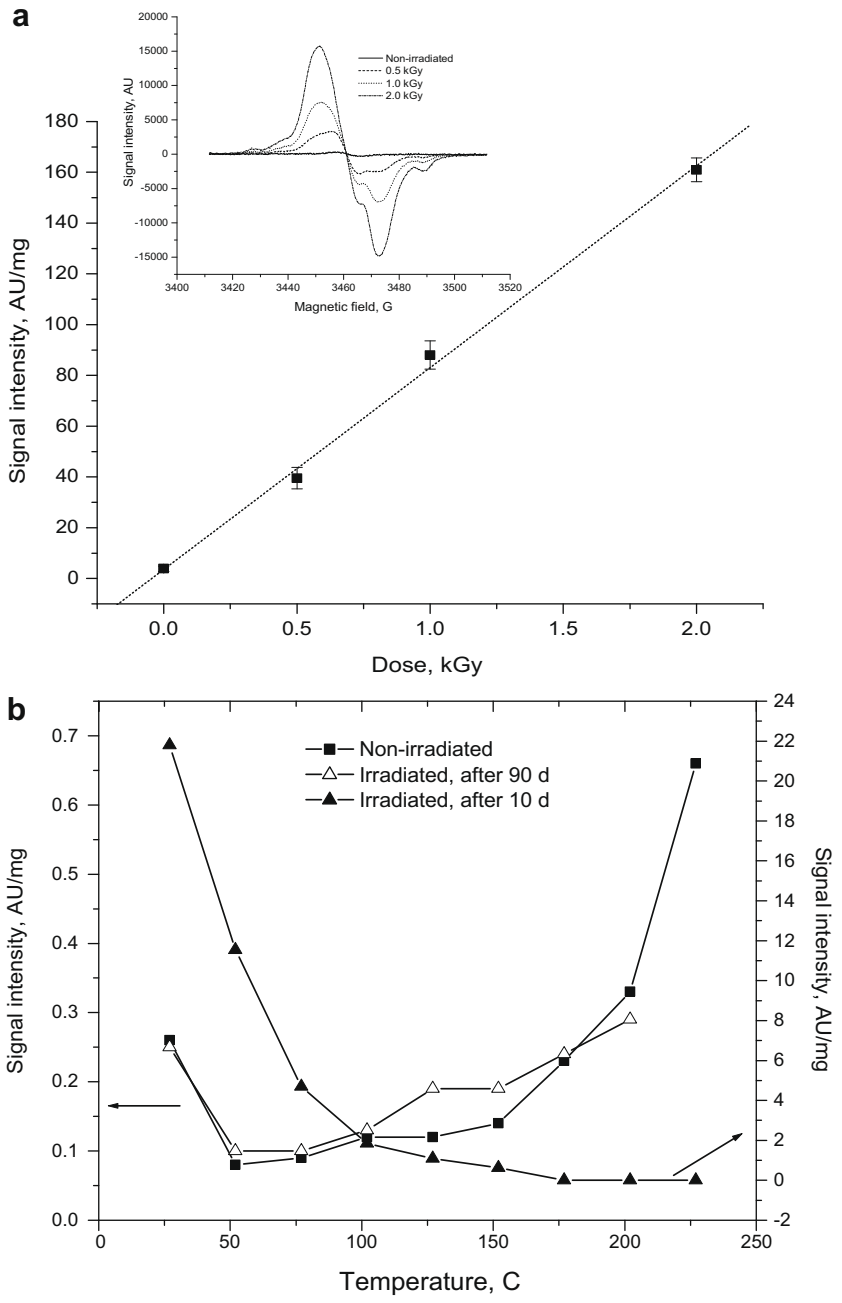

Fig. 2. (a) Response of the radiation induced signal intensity of the central line (g = 2.005) with increasing radiation dose and inset figure depicts the superposed EPR spectra of increasing radiation doses and (b) thermal behaviour of the central line of EPR spectrum for non-irradiated (control), irradiated (after 10 and 90 days) rice samples.

investigated as depicted in Fig. 1d. Irradiated starch exhibited similar signals as that of irradiated rice. This 'sugar-like' spectrum, originated from the radiation treatment of polysaccharides is considered to be an unambiguous evidence of the radiation treatment of the sample under investigation and recommended for the detection of irradiated foods in EN 13708 standards. The same has been validated for irradiated skin of raisins and figures containing considerable amount of polysaccharides in the form of sugar (EN 13708, 2001). These radiation specific signals observed in the rice matrix were not particularly stable and disappeared after 3–4 days with considerable decrease in intensity of the central singlet ($g = 2.005$) to a level that was similar to that of non-irradiated specimen. The instability in signal intensity means that the detection of irradiated rice by EPR spectroscopy is limited.

The effect of increasing radiation dose from 0.5 to 2.0 kGy on the spectra of rice samples was studied. The relative intensity of the central line was observed to be significantly related to the radiation dose as shown in Fig. 2a. The measurement was performed 2 days after irradiation. The dotted line represents a linear fit, $Y = aD + b$ with $a = 79.5$, $b = 3.5$ and each point representing the mean value of four samples.

3.2. Characterisations of non-irradiated and irradiated rice by thermal behaviour

The small life time of the radiation induced signals such as cellulose and 'sugar-like' strongly limits the applicability of the EPR analysis to detect irradiated food. This is the case with rice samples under investigation where the procedure based on EPR sugar-like signal (EN 13708, 2001) cannot be used beyond 2–3 days of radiation treatment. Therefore, alternative methods based on EPR are desirable. Thermal behaviour of the EPR signal was investigated in order to identify the irradiated rice samples from natural samples. Non-irradiated and irradiated samples were subjected to in situ heating from 27 to 275 °C. Fig. 2b shows the thermal behaviour of the non-irradiated and irradiated rice samples after a storage period of 10 and 90 days. The intensity of the central singlet of the irradiated rice after 10 days of storage exhibited a monotonous

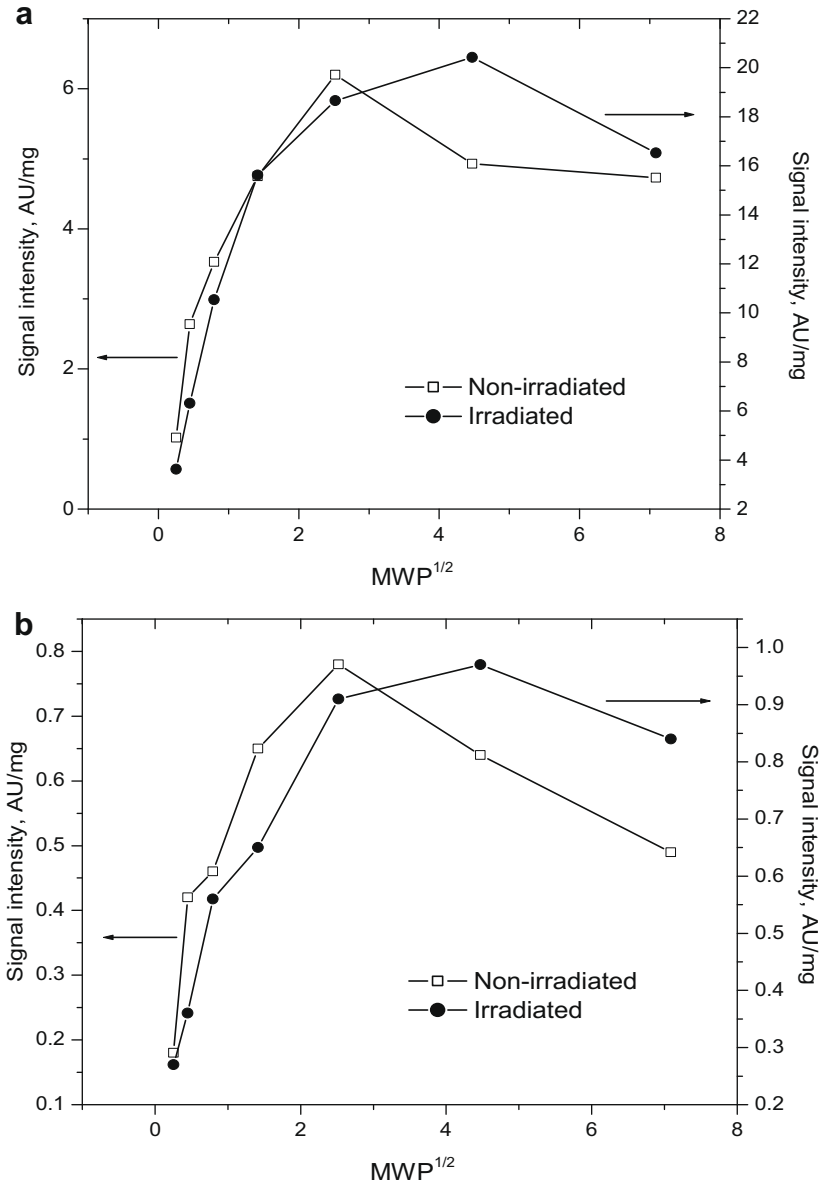

Fig. 3. Relaxation behaviour of main line of non-irradiated (control) and irradiated (1 kGy) rice sample (a) 10 days after irradiation and (b) 90 days after irradiation.

decrease in signal intensity up to 125 °C followed by almost unchanged behaviour up to 275 °C. Whereas, 90 days stored samples, both non-irradiated and irradiated (90 days) showed sharp fall in intensity of the central singlet up to 50 °C followed by slow increase up to 150 °C. However, temperature increases beyond 150 °C lead to an increase in signal intensities in both the cases. This could probably be due to the decomposition of the samples and formation of new thermally induced radicals. A decline of EPR signal of irradiated allspice sample has recently been reported by Polovka, Brezova, and Simko (2007). Yordanov and Gancheva (2000) proposed that EPR analysis of the central peak of spices that had undergone thermal treatment before and relatively long time after irradiation could be a tool for detection of irradiation. But, the present investigation revealed that a complete thermal behaviour of the singlet signal of rice samples can give a clue of radiation processing only after a storage period of 10 days. The thermal behaviours of the singlet of irradiated and non-irradiated rice samples after a long period of storage exhibited similar characteristics making the identification of irradiated rice sample difficult.

3.3. Characterisations of non-irradiated and irradiated rice by EPR saturation

The electron relaxation behaviours of radicals in the rice were studied. We varied the microwave field strength from 0.063 to 50 mW to obtain progressive saturation behaviour (PSB). The effect of saturation is manifested by continuous non-linear increase of EPR signal intensity with $P_{MW}^{\frac{1}{2}}$, reaching a maximum followed by a decrease with the simultaneous increase of EPR line width. Fig. 3a shows the PSB of the central line of non-irradiated and irradiated (1 kGy) samples 10 days after radiation treatment. For the radicals of non-irradiated rice sample a comparatively faster saturation at microwave power around 6 mW followed by a decrease in signal intensity in a monotonic fashion was observed. This saturation behaviour revealed the characteristics of organic radicals with large relaxation time. Whereas, the radicals of irradiated rice sample exhibited saturation at microwave power at around 20 mW. As depicted in Fig. 3b, even after a storage period of 90 days, the relaxation behaviour of non-irradiated rice sample exhibited early saturation of central singlet in comparison with the irradiated sample, similar to their behaviours noticed 10 days after radiation treatment. The EPR detection of irradiated dry food using microwave saturation has been proposed by Yordanov et al. (2006). In this method curves of saturation of non-irradiated and irradiated plants vs. $P_{MW}^{\frac{1}{2}}$ were studied and non-irradiated samples showed saturation at microwave power higher than 15 mW, whereas, irradiated sample exhibited early saturation at microwave power of around 8 mW. But, in the present study, non-irradiated rice samples exhibited early saturation even after a storage period of 90 days and revealed a significant difference in saturation behaviour from irradiated rice samples. As the EPR signals of cellulose or 'sugar-like' signals required by the European Standards were not visible in rice samples after prolonged storage, the application of Protocol EN 1787 or EN 13708 was not possible. However, faster EPR saturation of natural radicals in non-irradiated rice in comparison with the radiation induced radicals could be a tool to identify irradiated rice.

3.4. Kinetic study of the EPR singlet

Different factors, including humidity, temperature, light intensity, exposure to air and structure of the food matrix influence the behaviour of the induced radicals during storage of the sample

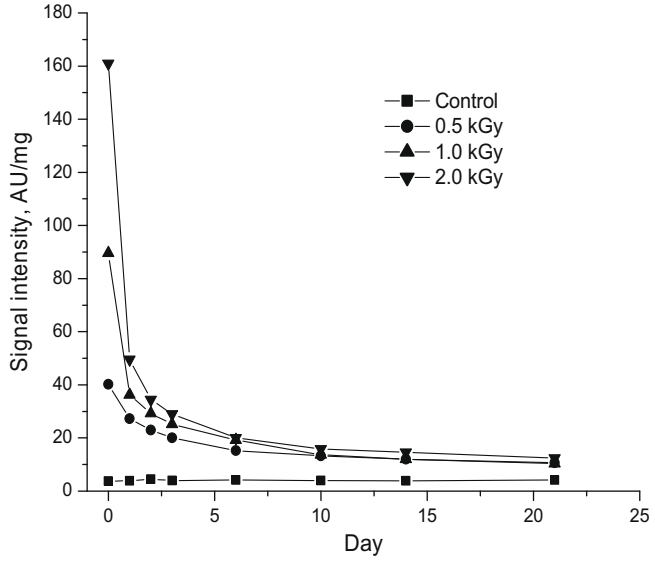

Fig. 4. Kinetics of the central line of the EPR signal of non-irradiated (control) and irradiated rice samples

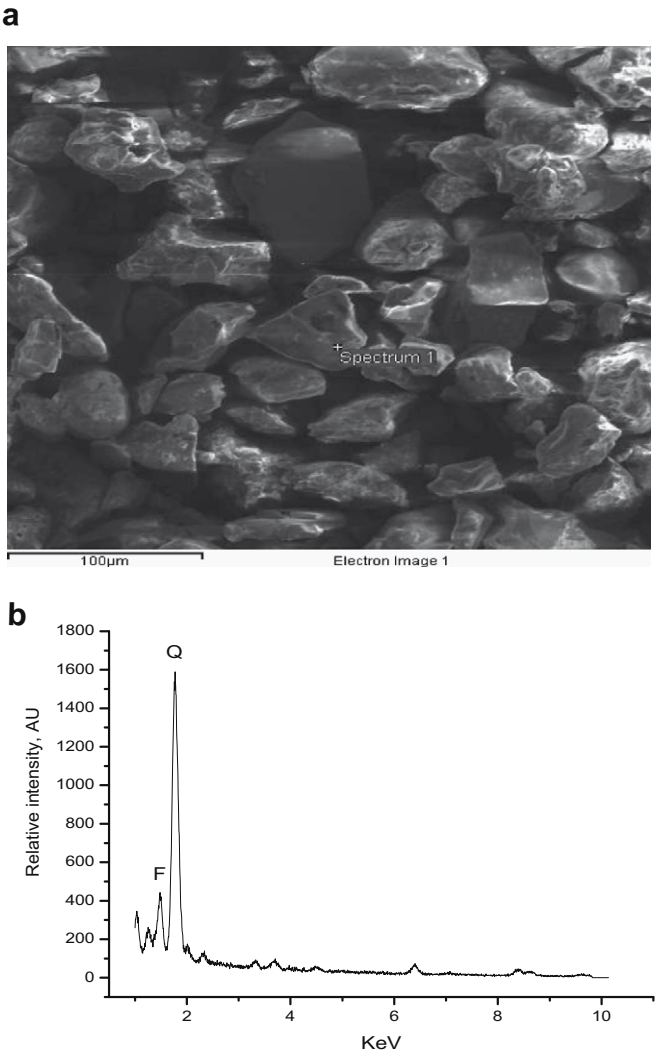

Fig. 5. (a) Scanning electron microscopy (SEM) image and (b) energy dispersive X-ray (EDX) spectrum of the poly-minerals extracted from rice. Feldspar: F and Quartz: Q.

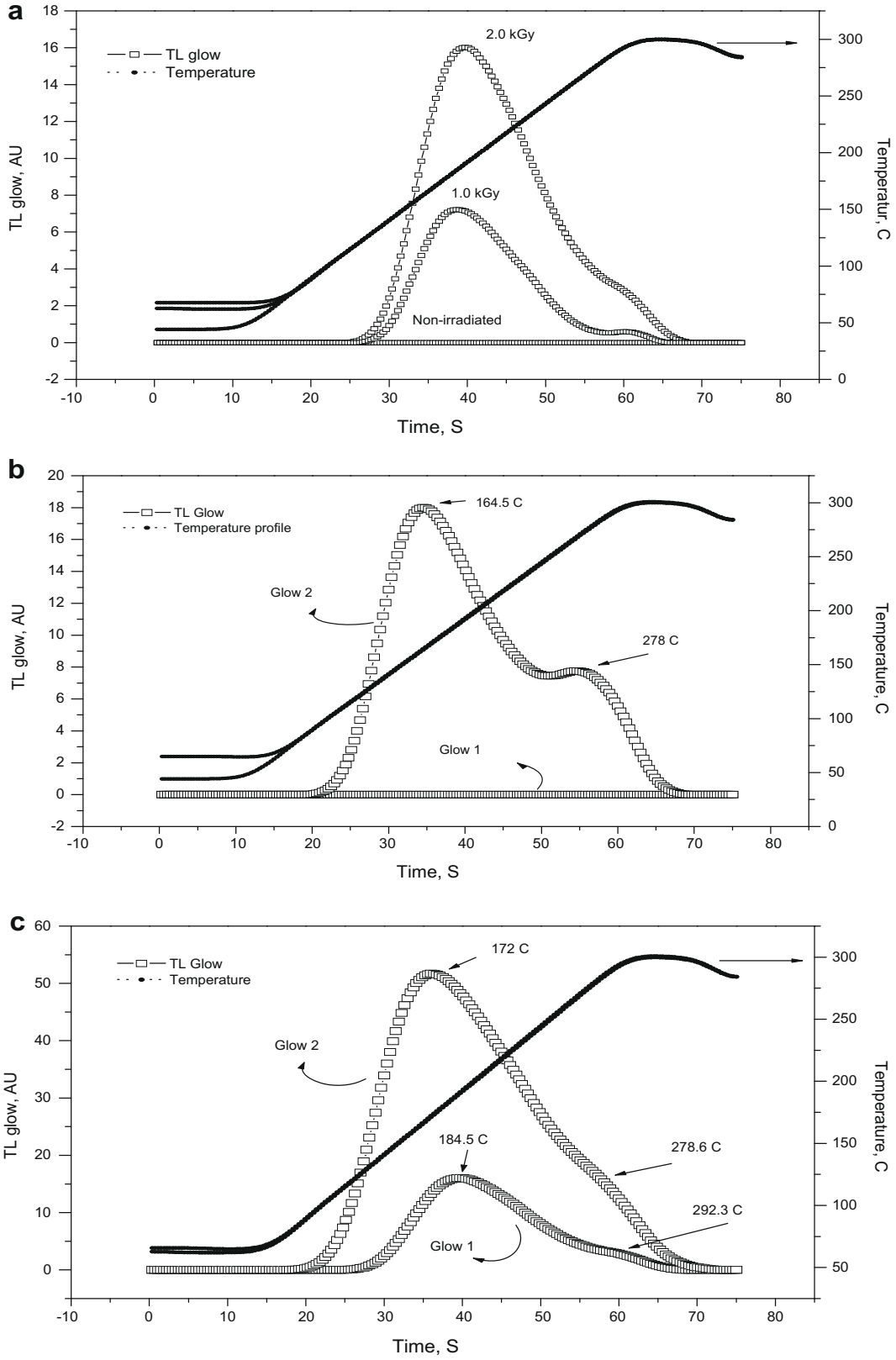

Fig. 6. (a) TL glows of isolated minerals from non-irradiated (control) and irradiated rice, (b) glow 1 and glow 2 of non-irradiated (control) sample and (c) glow 1 and glow 2 of irradiated sample.

before and after irradiation and thereby, restrict the time interval after irradiation during which detection is possible. 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 foods with time have been reported by many authors (Delincee, 1998; Raffi et al., 2000; Yordanov & Gancheva, 2000). In order to avoid these variations all the samples were kept inside the

EPR measurement tube under normal laboratory condition. Kinetics of non-irradiated and irradiated samples was monitored up to 12 weeks after radiation treatment. The radiation induced paramagnetic species identified as starch radical was observed to be stable up to 48–72 h after irradiation in the matrix of rice. Therefore, application of EN 1787 and EN 13708 Standards were not possible as a method of detection. Fig. 4 shows the fading characteristics of non-irradiated and irradiated rice samples throughout the storage period of 90 days. The results can be well fitted by a bi-exponential decay described by the following:

$$y = y_0 + A_1 \exp(-t/T_1) + A_2 \exp(-t/T_2)$$

where y represents the EPR signal intensity of the central line. Typical parameter values of the curve for the sample treated with 1 kGy radiation dose obtained with a non-linear least squares fitting procedure were $y_0 = 10.35$, $A_1 = 38.5$, T_1(days) = 3, $A_2 = 19.25$ and T_2 (days) = 15.

3.5. Thermoluminescence studies

TL method is applicable for the detection of irradiated foods, from which silicate minerals can be isolated (EN 1788, 2001). Therefore, investigation on the composition of the isolated minerals from the rice sample was of paramount importance to asses the possibility of employing TL method for the identification of the irradiated rice sample. In view of this, the composition of the separated poly-minerals from the rice sample was studied using scanning electron microscopy (SEM) and energy dispersive X-ray spectrometer (EDX) analysis. The results of these qualitative studies were interesting to examine the relative abundance of poly-minerals. Fig. 5a shows the SEM image of the extracted poly-minerals from the rice sample revealing the morphology. Fig. 5b shows the EDX spectrum which was mainly composed of quartz (SiO_2) and K-feldspars ($KAlSi_3O_8^-$) with a higher abundance of quartz (about 59.6%) than K-feldspars (20.7%). Apart from quartz and feldspar, traces of FeO (4.0%), Na_2O (4.1%), CaO (11.1%) were also identified. Similar patterns of mineral composition by X-ray diffraction (XRD) have been reported on herbs and spices such as oregano, mint and sage (Calderon et al., 1995), paprika (Correcher, Muniz, & Gomez-Ros, 1998), potato (Kwon, Jaeyoung, & Chung, 2002). TL response after radiation treatments is mainly responsible for quartz and feldspar component of the poly-minerals isolated from the food samples (Autio & Pinnoja, 1990).

Fig. 6a shows the TL intensities of glow curves for separated poly-minerals from the non-irradiated and irradiated rice samples 10 days after radiation treatment. In case of irradiated sample the glow curve was characterised by a low temperature peak at about 184 ± 4 °C and a high temperature peak at about 282 ± 5 °C. The position of glow peak for non-irradiated sample through all temperature ranges was not clear. However, low level natural radioactivity exhibited TL signal because of deep traps around 295 °C. The yield of the poly-minerals on each discs were in the range of 0.5–0.6 mg. The areas of glow curves for irradiated samples were 190–300 times more than the area from the non-irradiated samples. Higher values of TL glow (glow 1) in irradiated samples have been reported in previous studies on spices and herbs (Sanderson et al., 1996); chestnut (Chung et al., 2004). Glow peak temperatures at about 207 °C for irradiated anchovy and 192 °C for irradiated chestnut have been reported (Chung et al., 2004). Therefore, discrimination between irradiated and non-irradiated rice samples was possible on the basis of the shape of the first glow curve from the separated poly-minerals. Normalisation of results by re-irradiation with a dose of 1 kGy enhanced the reliability of the detection results. Fig. 6b and c shows the comparison of re-irradiation glow curves (glow 2) with respect to first glow curves (glow 1) for non-irradiated and irradiated samples respectively. The re-irradiation glow curves (glow 2) were characterised by a glow peak at 168 ± 4 °C and a higher temperature shoulder at 278 °C. The differences of the peak temperatures observed between glows 1 and 2 attributed to the time difference elapsed between irradiation and analysis because low energy trapped electrons were released during storage. Glow 2 was recorded 1 day after the normalisation doses whereas; samples were stored for several days after irradiation prior to the analysis of glow 1. The ratio of areas for first glow curve to second glow curve (glow 1/glow 2) determined for non-irradiated samples was 0.002 ± 0.023, while for irradiated sample 0.69 ± 0.085. Higher values of ratio of areas for glow 1/glow 2 in irradiated rice samples are in good agreement with the European Standard EN, 1788, 1997. TL glow of the irradiated samples were recorded after 65 days of storage in dark with normal laboratory condition and around 20% fading in TL glow intensity was observed with clear discrimination from the TL glow of the non-irradiated samples. Therefore, it was possible to discriminate clearly between irradiated and non-irradiated rice samples by the shape of the first TL glow and comparing the TL ratios (glow 1/glow 2).

4. Conclusions

Identification of gamma-irradiated Basmati rice was carried out by investigating free radicals using electron paramagnetic resonance (EPR) spectroscopy, and studying Thermoluminescence (TL) properties of the isolated poly-minerals. In case of EPR spectroscopy, a weak singlet was found in the commercially available rice sample. However, a short lived, complex spectrum was observed after radiation treatment attributed to radicals originating from starch. The change in free radical concentration showed a proportional increase with irradiation dose. By increasing the microwave power, the line shape of the EPR spectra altered and in saturation curves, it was possible to identify the irradiated and non-irradiated samples. However, thermal behaviour of the EPR signals was unable to identify irradiated rice samples. TL investigation of the poly-minerals isolated from the rice samples were carried out. SEM/EDX analysis revealed the maximum abundance of quartz (SiO_2) in the isolated minerals. TL glow curve structure of irradiated samples showed about 300 times increase in intensity than that of non-irradiated samples. Normalisation dose and ratio of the first and second glows (glow 1/glow 2) confirmed the identification of irradiated samples even after a long period of storage.

Identification of radiation treatment after storage of the sample under investigation was not possible by application of any of the European Standards (EN 13708; EN 1787) for EPR spectroscopy. TL technique provided promising results even after a long period of storage but, involved tedious sample preparation protocol (EN 1788, 2001; Carmichael & Sanderson, 2000). However, the microwave saturation characteristics of EPR signals of irradiated and non-irradiated rice can be a very useful tool to identify the irradiated sample from non-irradiated one even after prolonged period of storage.

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