CHAPTER 5

Identification of irradiated

Potato, Ginger, Fresh turmeric and Dog feed
Identification of irradiated potato using TL technique

The objective of the study was to identify potato of different geographical locations in India irradiated to sprout inhibiting dose, using TL characteristics of the isolated minerals. Potato (Chandramukhi) from two different districts, Bankura and Midnapore of West Bengal and markets of metro cities of Delhi and Mumbai were procured. The samples from each location were divided into three lots. One lot was kept as nonirradiated (control) and the remaining lots were irradiated with gamma radiation doses of 100 and 250 Gy. Minerals were isolated from both the nonirradiated and irradiated potatoes by density separation method. Characterization of the isolated minerals was carried out by X-Ray diffraction (XRD) technique to know the composition. XRD spectra of the isolated minerals from four different geographical locations are shown in Fig. 15 a – d. The XRD analysis of the polyminerals separated from potato showed that the relative abundance of K-feldspars (KAlSi$_3$O$_8$) and quartz (SiO$_2$) were predominant. However, a little variation in polymineral composition was observed with different origins of production. Similar patterns of mineral composition by X-ray diffraction (XRD) have been reported in herbs and spices such as oregano, mint and sage [102] and potato of South Korea [59]. TL response after radiation treatment is mainly responsible for quartz and feldspar component of the polyminerals isolated from food samples [104]. These findings revealed the possibility of using TL technique to identify irradiated potato.

TL glows were recorded from room temperature to 350°C at a heating rate of 5°C/s. The intensities of the glow curves for both the nonirradiated and irradiated samples showed significant difference even after a storage period of one month. Around 86 fold increases in TL glow (glow 1) of irradiated samples in comparison with nonirradiated ones was observed. Fig. 16 exhibited the TL glow of the irradiated and nonirradiated samples isolated from the potato procured from Bankura district of West Bengal after a storage period of 38 days. In all the cases, the glow curves (Glow 1) of irradiated samples were characterized by peak
temperature at around 204°C, whereas, the position of glow 1 for control sample through all temperature ranges was not clear. However, a small hump was observed at around 345°C, probably, because of the deep traps due to the presence of low level natural radioactivity in the environment.

Normalization dose of 250 Gy was delivered to all the samples and TL glow (glow 2) was measured with similar experimental settings. The reirradiation glow curve (glow 2) was characterized by a glow peak at 175°C as depicted in Fig. 16b. The differences of the peak temperatures observed between glow 1 and 2 are attributed to the time difference elapsed between irradiation and analysis because of low energy trapped electrons released during storage. The ratio of glow 1 / glow 2 for nonirradiated samples of all the geographical locations was 0.003±0.0005 and for irradiated samples it was 0.25±0.052. Higher values of ratio of areas for glow 1/glow 2 in irradiated potato samples are in good agreement with the European Standard EN 1788, 1996 [45]. In order to study the effects of increasing dose on the polyminerals, the potato samples were irradiated with 100, 250, 500 and 1000 Gy doses. Minerals were isolated from the potatoes by standardized density separation method to study the TL response. An increase in integral TL was observed with the increasing dose as depicted in Fig. 16c.

The results of the present study revealed that isolation of polyminerals from the surface of potato is possible using density separation and TL measurement can clearly differentiate between irradiated and nonirradiated samples. The reirradiation step improved the reliability of the detection results of TL.
Fig. 15. XRD spectra of the isolated minerals from the potato of different geographical locations a) Bankura, West Bengal, b) Midnapore, West Bengal, c) Delhi and d) Mumbai.
Fig. 16. a) TL glow curves of isolated minerals from nonirradiated and irradiated potato and b) TL glow 1 and glow 2 of the irradiated samples from Bankura district, West Bengal, c) TL glow curves of the isolated minerals from irradiated potato with increasing radiation dose.
Identification of irradiated ginger and fresh turmeric using TL technique

Identification of irradiated ginger (*Zingiber officinale*) and fresh turmeric (*Curcuma longa*) were studied using TL technique. Ginger and fresh turmeric were procured from a local market in Mumbai and distributed into three lots. One lot was kept as nonirradiated (control) and the remaining lots were irradiated with gamma radiation doses of 80 and 120 Gy. Minerals were isolated from both the nonirradiated and irradiated samples by density separation method. TL glow curves were recorded by heating the isolated minerals from room (27±2°C) temperature to 330°C at a heating rate of 5°C/s. The integral TL and the intensities of the glow peaks for both the nonirradiated and irradiated samples showed significant difference even after a storage period of one month. Around 50 fold increase in integral TL glow (glow 1) in irradiated ginger and around 20 fold increase in glow (glow 1) in case of irradiated fresh turmeric were observed in comparison with their respective nonirradiated samples. Fig. 17a and b show the TL glow curves of the irradiated and nonirradiated minerals isolated from ginger and fresh turmeric after a storage period of 35 days. In all the cases, the glow curves (Glow 1) of irradiated samples were characterized by a peak at temperature of 225±4°C and a small hump at a high temperature of 330±2°C, whereas, the position of glow curve 1 for control sample through all temperature ranges was not prominent.

Normalization dose of 1 kGy was delivered to all samples and TL glow (glow 2) was measured with similar experimental settings. The reirradiation glow curve (glow 2) for both the nonirradiated and irradiated samples isolated from ginger were characterized by an intense glow peak at 152±4°C and a high temperature hump at around 330°C as depicted in Fig. 18a and b. The reirradiation glow curve characteristic (glow 2) of the inorganic minerals isolated from the nonirradiated and irradiated fresh turmeric were also characterized by an intense glow peak at 152±4°C. In addition two more weak peaks were identified at 226±3°C and 331±1°C as shown in Fig. 18 c and d confirming the polyminerals nature of the isolated
samples. The differences in the peak temperatures observed between glow 1 and 2 were attributed to the time difference elapsed between irradiation and analysis. The low temperature peak of glow 2 at around 155°C in all the samples was recorded immediately after reirradiation dose of 1 kGy. The peak temperature suggested that this peak was responsible for shallow trapped electrons and was not stable during prolonged storage. Glow 1 (Fig. 17a, b) for all the samples were recorded after a period of one month, and therefore, a stable peak at around 225°C was observed. The ratio of glow 1 / glow 2 for nonirradiated ginger and fresh turmeric was negligibly small (0.00002). Whereas, in case of irradiated (80 Gy) ginger and fresh turmeric the same was measured as 0.02±0.0011 and 0.02±0.002, respectively. The glow curve structure (glow 2) of reirradiated samples confirmed the inorganic nature of the sample material. The higher values of glow1 / glow 2 in irradiated samples endorsed the effective detection of radiation treatment in good agreement with the European Standard EN 1788, 1996 [45]. In all the cases the higher dose of irradiation at 120 Gy exhibited increased level of TL output. The results of the present study revealed that isolation of polyminerals from the surface of ginger and fresh turmeric was possible using density separation, and TL measurement can clearly differentiate between irradiated and nonirradiated samples. The reirradiation step improved the reliability of the detection results of TL.
Fig. 17. TL glows (glow 1) of the isolated minerals from the irradiated and nonirradiated samples of a) ginger and b) fresh turmeric.
Fig. 18. Glow curve characteristics of glow 1 and glow 2 (normalized with 1 kGy) of the isolated minerals from a) nonirradiated ginger, b) irradiated (120 Gy) ginger, c) nonirradiated fresh turmeric, and d) irradiated (120 Gy) fresh turmeric.
Identification of irradiated dog feed

Exposure to ionizing radiation is an effective microbial disinfection process for animal feed. It is currently used in many countries for dog chew to control pathogens namely Salmonella. The irradiation of poultry feed for control of Salmonella is also approved by FDA in US. In India, commercial radiation processing of animal feed is carried out for microbial decontamination with an average dose of 7 kGy. Pet food manufacturers use dietary fiber sources from grains, fruits and vegetables, cellulosics, gums, and other sources. The main ingredients normally include cereal and cereal byproducts, meat and poultry industry waste, vegetable byproducts, proteins, vegetable oils, iodized salt, essential vitamins and minerals. In the present work, a detailed study of the radical species produced by gamma radiation in two varieties of dog feeds, namely, ready-to-eat granular form and edible dog chew in stick form has been carried out by EPR spectroscopy. The thermoluminescence technique has also been employed for confirmation of identification of radiation treatment of the samples. In addition, characterization of the extracted minerals from the edible dog chew samples was carried out for TL studies and the requirement of independent detection methods to give reliable identification of the irradiated samples has been established.

EPR spectroscopy on granular dog feed

Fig. 19 a depicts the EPR spectra recorded with microwave power 0.253 mW of nonirradiated and irradiated samples of granular form ready to eat dog feed. The EPR spectrum of nonirradiated sample was characterized by a singlet with $g = 2.0047 \pm 0.0003$ and $\Delta B_{pp} = 0.810$ mT. These free radicals were of semiquinones produced by the oxidation of polyphenolics [96] or lignin [97, 98]. Immediately after the radiation treatment of the sample at 7.5 kGy dose, a complex and broad EPR spectrum was observed with an increase in signal intensity of the existing weak singlet ($g = 2.0052 \pm 0.0002$). Increase in line width ($\Delta B_{pp}$) of the EPR signal from 0.766 to 1.311 mT could probably be attributed to the induction of multiple
paramagnetic centres in the matrix of dog feed. As shown in Fig. 19a, both nonirradiated and irradiated EPR spectra recorded with low microwave power of 0.253 mW, did not show any radiation specific signal. However, when the same samples were subjected to high microwave power of 50 mW, a visible change in the shape of the EPR spectrum was observed in case of irradiated sample as depicted in Fig. 19 b. The signal characterized by \( g = 2.0052 \) exhibited reduction in signal amplitude and an axially symmetric anisotropic signal with \( g_\perp = 2.0028 \) and \( g_\parallel = 1.9976 \) was identified. In order to characterize the radiation induced signal, EPR spectra simulation studies were carried out using WIN EPR and Simfonia programme (BRUKER). The detailed simulation scheme of EPR spectra of ready-to-eat granular form dog feed irradiated with a dose of 7.5 kGy is depicted in Fig. 20 a – c. Fig. 20 c exhibits the superposition of experimental and simulated spectra. The simulated spectrum was a linear combination of the individual signals represented in Fig. 20 a and b \( (R^2 = 0.962) \). Simulation of EPR spectra revealed the formation of two paramagnetic species. The isotropic signal was attributed to phenoxyl radical ion \( (C_6H_5O^-) \). The other anisotropic signal was probably because of \( CO_2^- \) radical ion formed due to breakdown of the fatty acid which is one of the major constituents of the chicken meat associated with ready-to-eat dog feed. The spin Hamiltonian parameters used to simulate phenoxyl radical ion were \( g = 2.0052 \) and \( \Delta B_{pp} = 0.86 \) mT and for anisotropic \( CO_2^- \) radical ion, \( g_\perp = 2.0028 \), \( g_\parallel = 1.9976 \) and \( \Delta B_{pp} = 0.30 \) mT. The \( g \) values obtained compare well with those reported in literature [109, 58]. The anisotropic signal of \( CO_2^- \) observed at 50 mW power was stable throughout the storage period of 90 d and could be considered as a marker for radiation treatment. In order to further reconfirm the nature of these radical species, their electron relaxation behavior was studied. Fig. 21a exhibits the EPR spectra recorded with a narrow scan width of 4 mT and exhibited the behavioral change in signal amplitudes of both the isotropic and anisotropic signals with the variation of the microwave field strength from 0.063 to 50 mW. The inset in Fig. 21a
shows the progressive saturation behavior (PSB) of the signals. The EPR signal at $g = 2.0052$ showed comparatively faster saturation at microwave power of 20 mW followed by decrease in signal intensity by monotonic fashion. The microwave saturation characteristics of these radicals suggested that they were possibly of organic origin with large relaxation time. However, the PSB of CO$_2^-$ radical ion signal showed a monotonous increase in signal intensity without any saturation confirming the characteristics of paramagnetic centre of inorganic origin and short relaxation time. The integral intensities of the EPR signals were obtained by double integration of the spectrum and the integral intensity of the central line was observed to be significantly related to the radiation dose as shown in Fig. 21b. The integral intensity showed saturation above 7.5 kGy radiation dose. The measurements were performed 2 d after irradiation.
Fig. 19. EPR spectra of nonirradiated (control) and irradiated (7.5 kGy) of ready to eat dog feed (granular form) immediately after irradiation recorded with microwave power a) 0.63 mW and b) 50.2 mW.
Fig. 20. Simulation scheme of the EPR spectrum of irradiated dog feed (granular form) a) simulated spectrum of phenoxy radical ion (C₆H₅O⁻), b) simulated spectrum of CO₂⁻ radical ion, and c) simulated and experimental spectra of irradiated dog feed.
Fig. 21. a). Superposition of EPR spectra of irradiated dog feed (granular form) recorded with increasing microwave power. The inset shows the progressive saturation behavior of the isotropic signal at $g = 2.0052$ and anisotropic signal at $g_{\perp} = 2.0028$, b) response of the radiation induced signal ($g = 2.0052$) and the dependence of double integral EPR signal intensity evaluated with increasing dose after 3 d of radiation treatment.
**EPR spectroscopy on gamma irradiated edible dog chew**

Fig. 22a depicts the EPR spectra of nonirradiated and irradiated dog chew (stick form) recorded at microwave power of 0.253 mW. Both the nonirradiated and irradiated samples were characterized by an isotropic signal $g = 2.0034$. No radiation specific signal was observed in irradiated sample. However, the signal amplitude of the isotropic signal increased with radiation treatment. The high power EPR spectra also did not provide any clue of radiation specific EPR line for this sample. A negligible change in edible dog chew after radiation treatment makes the identification of irradiated material an extremely challenging task. Therefore, European standards for detection of irradiated food by EPR spectroscopy released by European Committee of Normalization (CEN), namely food containing bone [EN 1786, 1997], cellulose [EN 1787, 2000] and crystalline sugar [EN 13708, 2001] cannot be employed to identify irradiated dog chew. In view of this, the electron relaxation behavior of radicals was studied. The inset in Fig. 22a shows the PSB of the central line of nonirradiated and irradiated (7.5 kGy) samples 90 d after radiation treatment. For the radicals of nonirradiated sample a continuous increase in signal intensity was observed with increasing microwave power, without any sign of saturation, revealing the inorganic nature of the paramagnetic centre. Whereas, in case of irradiated sample, a faster saturation at microwave power of 6 mW, followed by a decrease in signal intensity in a monotonic fashion was observed. This saturation behavior revealed the characteristics of organic radicals. The results of the present study are in good agreement with the EPR detection of irradiated dry food using microwave saturation as proposed by Yordanov et al, [100]. The integral intensities of the EPR signals were obtained by double integration of the spectrum and the integral intensity of the central line was observed to be significantly related to the radiation dose as shown in Fig. 22b. The measurements were performed 2 d after radiation treatment.
The time interval after which identification of the irradiated sample is possible was evaluated by the fading kinetics of the radiation induced radicals. In order to avoid background variations all samples were kept inside the EPR measurement tube under normal laboratory conditions. Fig. 23a shows the time kinetics of nonirradiated (g = 2.0047) and irradiated (g = 2.0052, g┴ = 2.0028) dog feed granules monitored up to 75 d after radiation treatment. In case of isotropic signal because of the organic radicals, a fast decrease in signal intensity was observed. Whereas, the anisotropic signal, because of CO₂⁻ radical ion observed at high microwave power, showed slower reduction in signal intensity and was stable during prolonged storage. Fig. 23b depicts the behavior of the EPR signal (g = 2.0034) of the nonirradiated and irradiated samples of edible dog feed during storage.
Fig. 22. a) EPR spectra of nonirradiated (control) and irradiated (7 kGy) of edible dog chew (stick form) immediately after irradiation recorded with microwave power of 0.63 mW, b) response of the radiation induced signal (g = 2.0034) and the dependence of double integral EPR signal intensity evaluated with increasing dose after 3 d of radiation treatment.

Fig. 23. Time kinetics of nonirradiated and irradiated samples of dog feed during storage a) for ready to eat granular form, b) edible dog chew stick form.
Thermoluminescence measurements of irradiated edible dog chew

A negligible change in the matrix of edible dog chew samples after radiation treatment makes the identification process using EPR spectroscopy a challenging task. However, the alternative approach based on relaxation behavior of the radiation induced paramagnetic centres provided a clue to radiation treatment. In order to give a reliable result thermoluminescence study of the nonirradiated and irradiated edible dog chew was carried out. Investigation on the composition of the isolated minerals from the edible dog chew sample was carried out using scanning electron microscopy (SEM) and energy dispersive X ray spectrometer (EDX) analysis to assess the possibility of employing TL method for the identification of the irradiated sample. The results of these qualitative studies were interesting to examine the relative abundance of polyminerals. Fig. 24a shows the SEM image of the extracted polyminerals. Fig. 24b shows the EDX spectrum and weight percentage of the major constituents namely calcium (Ca), silicon (Si) and iron (Fe). Calcium being the major component of meat bone associated with the edible dog chew may be responsible for TL signal. Silicon in the form of quartz and aluminum as feldspar are the other two important contributors of TL in this sample.

Fig. 24c shows the TL intensities of glow curves for separated polyminerals from the nonirradiated and irradiated edible dog chew 7 days after radiation treatment. In case of irradiated sample the glow curve was characterized by a low temperature peak at about 213±3°C and a high temperature peak at about 303±5°C. The low temperature peak height (P_{1\text{height}}) and high temperature peak height (P_{2\text{height}}) represented the TL intensities at the corresponding glow peak temperatures. No glow peak through the entire temperature range was identified for nonirradiated sample. However, low level natural radioactivity exhibited TL signal because of deep traps around 303°C. The areas under the glow curves for irradiated sample (10 kGy) were 55 times more than the areas under the nonirradiated sample. Higher
values of TL glow (Glow 1) in irradiated samples have been reported in previous studies on spices and herbs [105], chestnut [64]. Therefore, on the basis of the shape of the first glow curve from the separated polyminerals, discrimination between irradiated and nonirradiated samples was possible. Fig. 24d exhibits the dose dependent response of the TL glows of the isolated polyminerals from the edible dog chew. An increase in intensities (P_{1height}) of the low temperature peak (213°C) with increasing dose was observed. Normalization of results by re-irradiation with a dose of 1 kGy enhanced the reliability of the detection results. Fig. 25a and b show the comparison of reirradiation glow curves (glow 2) with respect to first glow curves (glow 1) for nonirradiated and irradiated samples, respectively. The reirradiation glow curves (glow 2) were characterized by three glow peaks. The first peak was at 148±3°C, the second and the third peaks were at 213±2°C and 306±5°C, respectively. The differences of the peak temperatures observed between glow 1 and 2 attributed to the time difference elapsed between irradiation and analysis. Glow 2 was recorded 1 d after the normalization doses, whereas, samples were stored for several days after irradiation prior to the analysis of glow 1. In case of nonirradiated sample the ratio of glow 1 / glow 2 was 0.035±0.003, while for the samples irradiated at doses 2.5, 5, 7.5 and 10 kGy the ratios were found to be 0.930±0.012, 2.169±0.25, 2.48±0.22 and 1.90±0.32, respectively. The higher values of the ratio of areas for glow 1/glow 2 for irradiated samples are in good agreement with the European Standard EN 1788, 1996 [45]. The dog chew samples (irradiated and nonirradiated) were stored for seven months to study the fading kinetics of the TL glows. During prolonged storage the isolation of minerals from nonirradiated and irradiated samples was carried out prior to each TL measurement. Fig. 25c and d exhibit the TL glow curve structures of the 2.5 and 10 kGy irradiated samples, respectively. In both the cases the P_{1height} showed a fast decrease in TL intensity with time, whereas, the P_{2height} revealed slow reduction in intensity. The insets of the figures show the variation of the ratio of P_{1height} to P_{2height} (P_{1height} / P_{2height}) with increasing
time and confirmed the fast fading kinetics of $P_{\text{height}}$. However, a clear discrimination between irradiated and nonirradiated edible dog chew samples was possible from the shape of the first TL glow even after a prolonged storage of seven months.
Fig. 24 a) Scanning Electron Microscopy (SEM) image, b) Energy Dispersive X ray (EDX) spectrum of the polyminerals extracted from dog chew samples, c) TL glows of isolated minerals from nonirradiated (control) and irradiated (10 kGy) edible dog chew (stick form) d) response of the TL glows of the isolated minerals with increasing radiation doses from 2.5 to 10 kGy.
Fig. 25. TL glows of isolated minerals from dog chew samples a) glow 1 and glow 2 (normalized with 1 kGy) of nonirradiated (control) sample and b) glow 1 and glow 2 (normalized with 1 kGy) of irradiated (10 kGy) sample. Response of the TL glows during storage for the samples subjected to radiation doses c) 2.5 kGy and d) 10 kGy and insets show the behavior of \( P_{1_{\text{height}}} / P_{2_{\text{height}}} \) with increasing time.