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Analytical Methods

Detection of irradiated black tea (*Camellia sinensis*) and rooibos tea (*Aspalathus linearis*) by ESR spectroscopy

Mustafa Polat *, Mustafa Korkmaz

Department of Physics Engineering, Hacettepe University, 06800 Ankara, Turkey

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Abstract

Detailed ESR investigation on irradiated black and rooibos tea was carried out in the dose range of 0.5–10 kGy. Unirradiated black and rooibos tea samples exhibit a weak, symmetric ESR (electron spin resonance) singlet centered at $g = 2.0043 \pm 0.0010$ with peak-to-peak line widths (ΔH_{pp}) of 1.00 ± 0.05 and 0.64 ± 0.05 mT, respectively. Irradiation caused a significant increase in ESR signal intensity of both samples without any changes in spectral patterns and these increases were found to be explained by a quadratic and/or an exponentially varying functions. Variation of ESR signal intensity, for the irradiated samples, at room temperature (295 K) with time in a storage period of 39 days showed that free radical responsible from the ESR spectrum of black tea is more stable than that of the rooibos tea. However, variable temperature and annealing studies show that the free radical responsible from the ESR spectrum of rooibos tea, with activation energy of 46.0 ± 3.5 kJ mol^{-1}, is more resistant to the temperature than that of the black tea with activation energy of 33.8 ± 3.1 kJ mol^{-1}.

Keywords: ESR; Irradiation; Kinetic; Tea; Rooibos

1. Introduction

Tea is one of the most commonly consumed beverages throughout the world. Various kinds of tea are consumed, but almost all of them are produced from the same species of plant, *Camellia sinensis* (Harold & Graham, 1992; Muthumani & Kumar, 2007). Based on the manufacturing technique, teas can be classified as: green-tea (20–22% of world tea consumption), Oolong-tea (2–3% of world tea consumption) and black tea (73–78% of world tea consumption) (Krishnan & Maru, 2006). The leaves and fine stems of the leguminous shrub *Aspalathus linearis*, known as rooibos, are used to produce rooibos tea, a beverage that is becoming increasingly popular due to its unique taste, versatility and, most importantly, its reputation as a health drink. This reputation stems from claims of antioxidant activity and its therapeutic and physiological advantage (Jaganyi & Wheeler, 2003). Normal consumption involves brewing the leaves and then consuming the liquor hot or cold. It is often used as an ingredient in various recipes (Jaganyi & Wheeler, 2003).

Many processing methods have been developed to help prevent food spoilage and improve safety. The traditional methods of preservation, such as drying, smoking and salting have been supplemented with pasteurisation, canning, refrigeration, freezing and chemical preservatives (Kader, 1986). Treatment of food with X-rays, γ-rays, or electron-beam irradiation with the aim of improving its shelf-life and hygienic quality is becoming increasingly important in the growing number of countries and has increased enormously during the past decades.

Development of analytical methods to detect irradiated foodstuffs is very important from the point of view of regulation and consumer confidence. A range of analytical methods have been successfully developed. These methodologies complement each other and allow clear discrimina-

* Corresponding author. Fax: +90 312 299 20 37.
E-mail address: polat@hacettepe.edu.tr (M. Polat).

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tion between irradiated and unirradiated products in a wide variety of foods. ESR is one of the leading methods for identification of irradiated foodstuffs (Korkmaz & Polat, 2000, 2001; Raffi & Stocker, 1996). ESR yields both qualitative information (i.e. whether or not a sample has been irradiated) and quantitative results (i.e. the dose it received).

Many research groups have reported the antioxidative properties of teas and rooibos (Jaganyi & Wheeler, 2003; Luczaj & Skrzydlewska, 2005; Morsy & Khaled, 2002; Von Gadow, Joubert, & Hansmann, 1997). However, there were little works related to ESR spectroscopic features of radiation-induced radical species in these samples. Therefore more detailed information, especially related to the radical stabilities at room and higher temperatures, are needed. The aim of the present work was: to test the ability of ESR technique to distinguish irradiated and unirradiated black and rooibos tea; to investigate the radical kinetics at room and high temperatures and finally, to construct the dose–response curve and microwave saturation features and discuss the possible use of black and rooibos tea as intrinsic dosimeters.

2. Materials and methods

Samples of wet black tea leaves harvested in 2004 (Rize, Turkey) and rooibos tea provided from local markets were used in this study. Wet black tea leaves were cleaned several times with water and dried at room temperature (295 K) for one week and then, were ground mechanically and sieved using 0.5 mm holes while rooibos tea was used as provided. All the samples were transferred to polycarbonate vials and were kept at room temperature, under constant atmospheric pressure and 30% relative humidity, for two weeks, before irradiation treatment, to permit the decay of mechanically-induced radical species. All irradiation and ESR experiments were carried out on samples open to air in order to stay under commercial food irradiation conditions and to determine the possible dosimetric use of the studied tea samples as a normal and/or accidental dosimeter. Irradiations were performed at room temperature using a ^{60}Co gamma cell, supplying a dose rate of 1.7 kGy/h as an ionizing radiation source at the Sarayköy Establishment of the Turkish Atomic Energy Agency in Ankara. The dose rate at the sample sites had been calibrated by Fricke dosimeter (ferrous sulphate dosimeter). Black and rooibos tea samples irradiated to doses of 0.5, 1.0, 2.0, 5.0, 7.0 and 10.0 kGy were employed to construct the dose–response curves. The uncertainty in radiation doses was nearly 5%. A variable temperature study on the samples irradiated at a dose of 5.0 kGy were performed with a temperature increment of 20 K in a temperature interval of 120–380 K by recording the spectra 5 min after each temperature setting. A long-term radical decay features at room temperature was also performed over a time period of ~39 days using the samples irradiated at a dose of 5.0 kGy. Decay kinetics of the radiation-induced free radical species at high temperatures (313, 333, 353, 373, 393 and 413 K) was performed by using the samples irradiated at a dose of 5.0 kGy. The black and rooibos tea samples were transferred after irradiation process to water baths at temperatures of 313, 333, 353, 373, 393 and 413 K then their ESR spectra were recorded regularly over a time interval of 0–60 min after cooling them to room temperature following predetermined heating times (2, 4, 6, 10, 20, 40 and 60 min). All spectral evaluations were performed by comparing the results with those obtained from spectra recorded at room temperature before heating. ESR measurements were performed with a delay of 1 h after stopping irradiation due to the distance between the irradiation plant and ESR spectrometer. Thus, we were not able to investigate radical or radicals of short half-lives in the present work.

ESR measurements were carried out using a Bruker EMX X-band ESR spectrometer operating at 9.5 GHz and equipped with a high sensitive cylindrical cavity (conditions of operation: central field: 347 mT; scan range: 20 mT; microwave power: 0.5 mW; microwave frequency: 9.791 GHz; receiver gain: 2.5×10^4; modulation frequency: 100 kHz; modulation amplitude: 0.1 mT; time constant: 327.68 ms; sweep time: 83.886 s). Sample temperature inside the microwave cavity was monitored with a digital temperature control unit (Bruker ER 4111-VT). The latter gave us the opportunity of measuring the temperature with an accuracy of ± 0.5 K at the site of the sample. A crystalline DPPH (1,1-diphenyl-2-picryl-hydrazyl) sample was used as a standard sample. Each measurement corresponds to the average of at least three different samples. The position of the sample in the cavity was not changed during the long-term signal intensity decay experiment to avoid any error in g factor and intensity measurements arising from changes in the cavity-filling factor.

3. Results and discussion

3.1. Room temperature ESR spectra of unirradiated and irradiated samples

Unirradiated black and rooibos tea samples (control) exhibit a weak, symmetrical ESR singlet both centered at $g = 2.0043 \pm 0.0010$ with peak-to-peak line widths (ΔH_{pp}) of 1.00 ± 0.05 and 0.64 ± 0.05 mT, respectively. Irradiation produces a significant increase in signal intensity of both samples. However, there were not observed any changes in g factors and line widths. Two weak satellite peaks on the sides of the main ESR resonance signal with a separation of 6 mT were also observed for rooibos tea samples irradiated at a dose of 10 kGy and can be attributed to the cellulose-derived radical. Similar ESR spectra were observed for a wide range of foods in the literature (Bayram & Delince'e, 2004; Bortolin, Griffin, Cruz-Zaragoza, De Coste, & Onori, 2006; Desrosiers, 1996; Korkmaz & Polat, 2001) The main ESR resonance signals of irradiated black and rooibos tea samples can be responsible from

the free radical species of different origin. Although their g values are the same, peak-to-peak line widths are significantly different. In case of black tea sample, the main ESR singlet can be responsible from a doublet with unresolved hyperfine structure. This singlet was observed in various foods and is attributed to the semiquinone radical in literature (Jezierski et al., 2002; Morsy & Khaled, 2001; Polovka, Brezová, & Staško, 2003; Suhaj, Racova, Polovka, & Brezova, 2006). It can be summarized that the contributing radicals to the ESR spectra of irradiated rooibos tea are semiquinone and cellulose-derived radicals. However, the contributing radical to the ESR spectra of irradiated black tea is semiquinone radical. Typical ESR spectra recorded for unirradiated and irradiated rooibos tea are given in Fig. 1a and b, respectively. The ESR spectrum of irradiated black tea sample was not given here for simplicity. The ESR signal intensities were used to follow the evolution of ESR spectra as a function of absorbed dose, temperature, and time. Unirradiated and irradiated (5.0 kGy) black and rooibos tea samples were found not to saturate up to a microwave power of 1.5 mW at room temperature (Fig. 2). As it is seen from this figure, microwave saturation features of the resonance signals of black and rooibos tea samples are quite different. This is also shows that the origin of free radicals induced in gamma irradiated black and rooibos tea is different. A microwave power of 0.5 mW was adopted throughout the experiment to avoid any saturation effect even at 120 K which is the lowest achievable temperature in the present work.

3.2. Dose–response curve

Samples of black and rooibos tea irradiated to doses of 0.5, 1.0, 2.0, 5.0, 7.0 and 10.0 kGy were used to construct the dose–response curves. Signal intensity variations with the absorbed radiation doses were given in Fig. 3. It is important to emphasize that, in the studied dose range of (0.5–10.0 kGy), the g factors and line widths do not change nor do other lines appear. Several mathematical functions

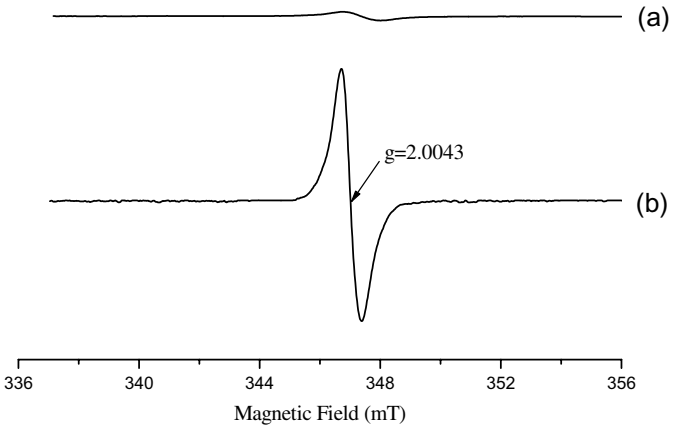

Fig. 1. Typical ESR spectra recorded for unirradiated and irradiated, at a dose of 5.0 kGy, rooibos tea samples. (a) Unirradiated and (b) irradiated.

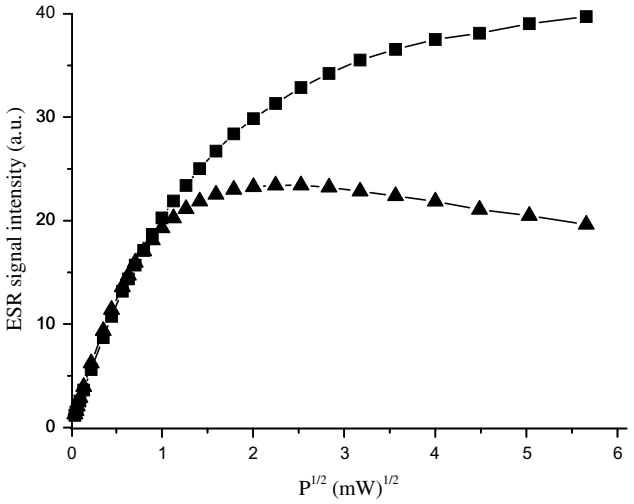

Fig. 2. Variation of ESR signal intensity with applied microwave power for the samples irradiated at the dose of 5.0 kGy. (■) black tea and (▲) rooibos tea.

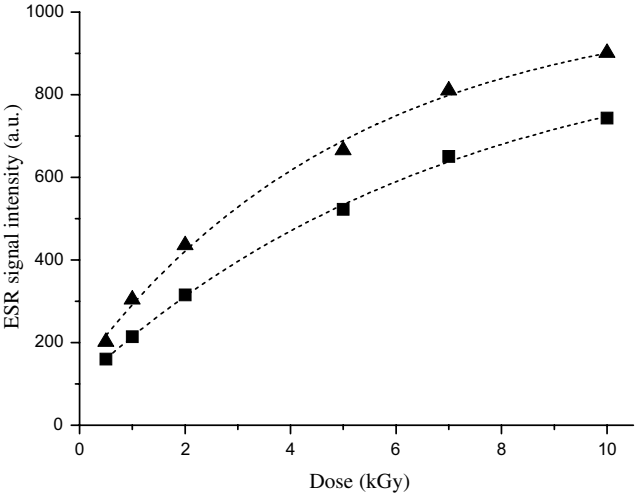

Fig. 3. Variation of ESR signal intensity with applied radiation dose (experimental: (■) black tea and (▲) rooibos tea; theoretical, calculated using exponentially varying function: dashed lines).

such as $Y = a + b * D$, $Y = a + b * D + c * D^2$, $Y = a + b * [1 - e^{-c*D}]$ and $Y = a + b * D^c$ were tried to fit experimental dose–response data to describe the ESR signal intensity variations with absorbed radiation dose for both samples. In these functions Y and D stand for the ESR signal intensity and absorbed radiation dose in kGy, respectively. The intercepts (parameter a) in these function represent the ESR signal intensity at zero applied radiation dose means that the ESR signal intensity of unirradiated (control) black and rooibos tea. Parameters b and c represent the rate of radical production and/or radiation-yield upon irradiation. If the intercept values (parameter a) calculated from these functions together with the correlation coefficients are taken into consideration, the dose–response curves of black and rooibos tea are explained best by exponentially varying functions. The parameter values

of a, b and c calculated from fitting procedures for black tea were found to be 102.6 ± 11.2, 855.0 ± 50.0 and 0.14 ± 0.02 ($r^2 = 0.9989$), respectively, and in case of rooibos tea, these values were calculated as 134.7 ± 23.9, 897.0 ± 52.6 and 0.19 ± 0.03 ($r^2 = 0.9968$), respectively. The theoretical dose–response curves calculated by using the parameter values given above for exponentially varying function were also given in Fig. 3 (dashed lines).

3.3. Variable temperature studies of irradiated samples

The variations of the ESR signal intensity, g factor and peak-to-peak line width (ΔH_{pp}) *vs.* temperature were also studied in the range of 120–380 K using the sample irradiated to a dose of 5.0 kGy. Cooling the samples from 290 to 120 K caused increases of ~80% and ~60% in ESR signal intensities of black and rooibos tea, respectively, but not significant changes in the g and ΔH_{pp} values. When the samples were heated from 120 to 290 K, the ESR signal intensities were found to follow the same paths, in a reversible way, and reached approximately their initial values before cooling. Heating the samples over 290 K caused significant decreases in the ESR signal intensities of both samples and reaching to a minimum value at 380 K, which is the highest achievable temperature in the present work. Heating the samples to higher temperatures (up to 380 K) caused decreases of ~76% and ~56% in ESR signal intensities of black and rooibos tea, respectively. Pre-cooling the samples from 380 to 290 K created relatively small increases in ESR signal intensities of both samples compared with those obtained at 380 K but, never reached to their before heating values at 290 K. Although, heating created changes in ESR signal intensities, it did not produce significant changes in other spectral parameters such as g factor and line widths of both samples. The decreases in ESR signal intensities at high temperatures were appreciated as serious decreases in the population of radiation-induced free radicals in the samples. Observed variations of ESR signal intensities at low and high temperatures for the samples irradiated at 5.0 kGy was given in Fig. 4.

3.4. Long-term radical decays at room temperature

The variations of ESR signal intensities *vs.* time was studied over a storage period of ~39 days using the samples irradiated at room temperature at a dose of 5.0 kGy. The samples were stored at room temperature (295 K) under normal conditions and their ESR spectra were recorded periodically during the storage period. Variation of the ESR signal intensities of black and rooibos tea samples was given in Fig. 5. The ESR signal intensities of both samples were found to decrease very rapidly in the first 20 days of storage then, the decreases became slower. It is important to emphasize that, during the storage period, g factors and peak-to-peak line widths of both samples do not change nor do other lines appear. However, even at the end of the storage period, ESR signal intensity values

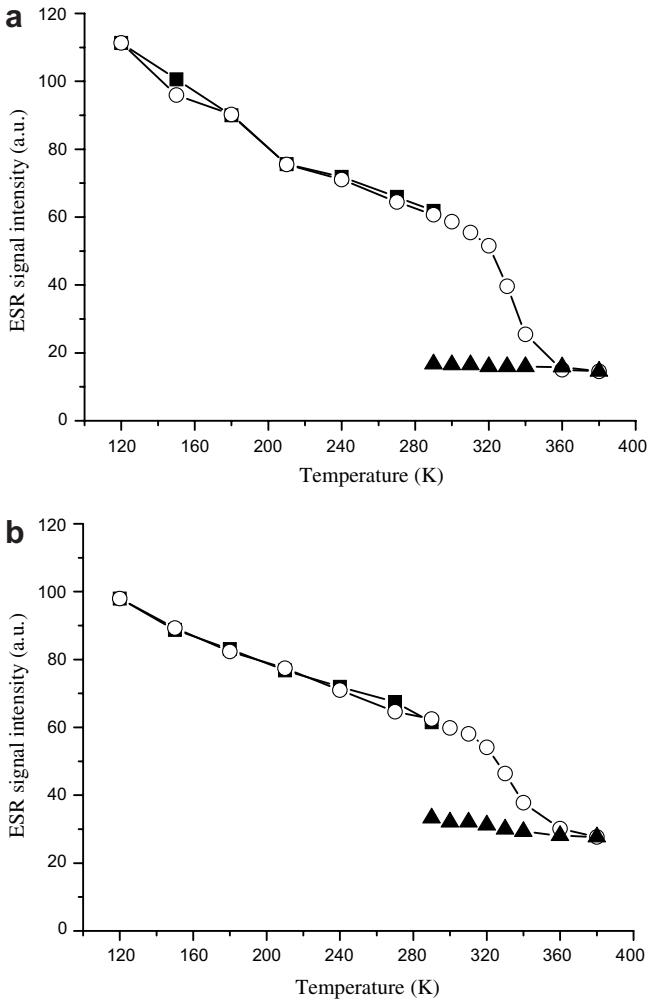

Fig. 4. Variation of ESR signal intensity with temperature for the samples irradiated at room temperature to a dose of 5.0 kGy. (a) Black tea, (b) rooibos tea. (■) Cooling (290 → 120 K), (○) heating (120 → 380 K) and (▲) cooling (380 → 290 K).

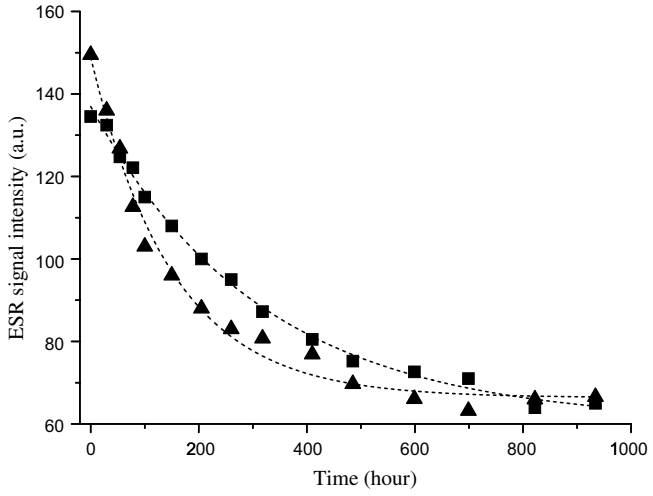

Fig. 5. Experimental and calculated ESR signal intensity decay curves for the samples irradiated at a dose of 5.0 kGy and kept at room temperature (experimental: (■) black tea and (▲) rooibos tea; therotical, calculated by a function describing second-order kinetic: dashed lines).

of the irradiated samples were found to be still fourfold higher than that of the control (unirradiated) samples. A function describing second-order kinetic $n = n_0/(n_0kt + 1)$ was used to fit the long-term signal intensity decay data given in Fig. 5. In this function n and n_0 represents signal intensity at any time and at zero, respectively, and k represents rate constant. The rate constants calculated by this procedure was found to be $3.18 \times 10^{-3}\,h^{-1}$ and $6.61 \times 10^{-3}\,h^{-1}$ for black and rooibos tea, respectively. The calculated decay data by using the rate constants values were also given in Fig. 5 as dashed lines.

3.5. Annealing at high temperatures

The fast decreases of ESR signal intensities at high temperatures (Fig. 4) can be attributed to the decreases of free radical populations in the sample due to the recombination of the radiation-induced free radicals and these decreases are strongly depends on the annealing time and temperature. Furthermore, it must be in mind that temperature itself can be a potential source of producing new radical species in the samples. To go more insight to the ESR signal intensity decay at high temperature, a set of black and rooibos tea samples irradiated at a dose of 5.0 kGy were annealed at six different temperatures (313, 333, 353, 373, 393 and 413 K) for predetermined times (2, 4, 6, 10, 20, 40 and 60 min). Although the samples were annealed at high temperatures, all ESR spectra were recorded at room temperature after cooling the samples. Variations of ESR signal intensities of black and rooibos tea samples were given in Fig. 6a and b, respectively. As it is expected, the higher the temperature and/or the annealing time the faster the decay of the signal intensities. At high temperatures, this decay was so fast that, at 413 K, the decreases of ~80% and ~67% in ESR signal intensities of black and rooibos tea, respectively, occurred over an annealing time of 6 min. Significant increases in ESR signal intensities were also observed at high temperatures (>393 K) after a critical annealing times (Figs. 6a and b) as in cereals, legume seeds and red pepper (Korkmaz & Polat, 2001; Polat & Korkmaz, 2003a, 2003b) and the higher the annealing temperature, the shorter this critical annealing time. The increases in ESR signal intensities were found to originate from the generation of new radicals in the samples. However, the characteristic features of these new radicals were found to be very similar to those of the original free radicals present in untreated samples. Because of the complexity of the experimental data obtained at high annealing temperatures (393 and 413 K), only the rate constants (k) of the free radical species at annealing temperatures of 313, 333, 353 and 373 K were calculated by fitting the experimental data to an expression derived by integration of the differential equation describing a second-order kinetic behavior (Masterton & Slowinski, 1969). Theoretical decreases in ESR signal intensities, calculated using the rate constant values

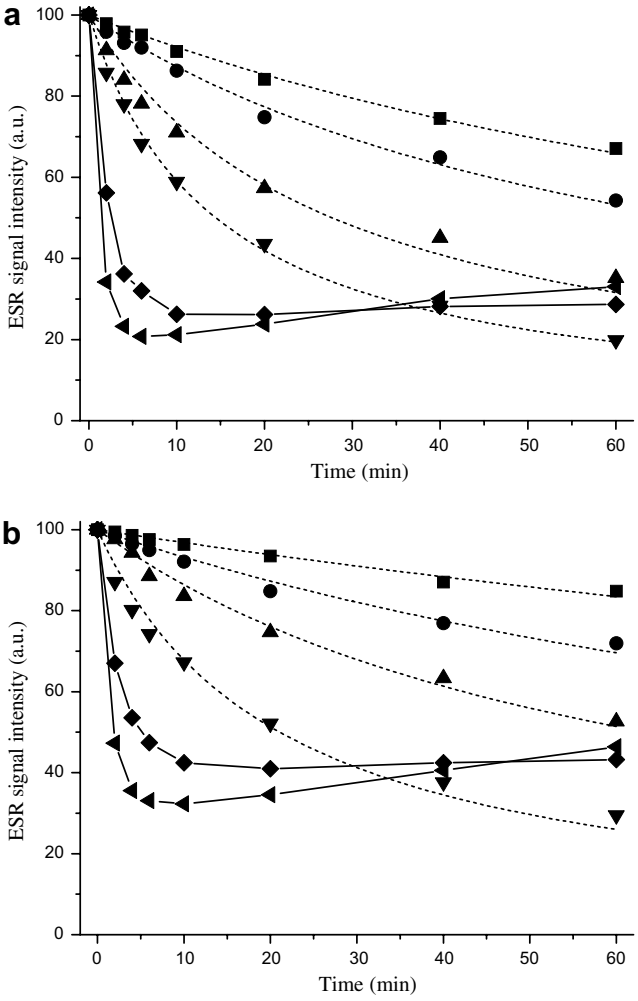

Fig. 6. Variations of the ESR signal intensity with annealing time for samples irradiated at a dose of 5.0 kGy and kept at different temperatures. (a) Black tea, (b) rooibos tea (experimental: (■) 313 K; (●) 333 K; (▲) 353 K; (▼) 373 K; (♦)393 K and (◀) 413 K; theoretical, calculated by a function describing second-order kinetic: dashed lines; data connection: solid lines).

determined after the fitting procedure, are also given in Figs. 6a and b (dashed lines) with their experimental counterparts. As can be seen from these figures, the decreasing calculated and experimental signal intensity data agree fairly well.

The rate constants (k) are expected to exhibit an exponential dependence on temperature of the type $k = k_0 \exp(-E_a/RT)$, where E_a is the reaction activation energy, R the gas constant and T the absolute temperature (Masterton & Slowinski, 1969). If so, a $\ln(k)$ *vs.* $1/T$ plot should give a straight line, the slope of which is proportional to the reaction activation energy. The reaction activation energy values of 33.8 ± 3.1 and $46.0 \pm 3.5\,kJ\,mol^{-1}$ were calculated from this plots for the free radical species contributing to the ESR signal of irradiated black and rooibos tea, respectively. This result shows that the free radical responsible from the ESR signal of irradiated rooibos tea is more stable than the free radical of irradiated black tea.

4. Conclusion

Unirradiated black and rooibos tea samples exhibit a weak, symmetric ESR singlet. Irradiation at room temperature (295 K) caused a significant increase in ESR signal intensities of both samples without any changes in g factors and peak-to-peak line widths. Two weak satellite peaks, with a separation of 6 mT, on the sides of the main ESR signal was also observed for rooibos tea irradiated at a dose of 10 kGy. The ESR signal intensity was found to depend on the absorbed radiation dose and increased with the increasing of radiation dose for both samples. The exponentially varying functions described best these variations. From the analysis of the data relevant to dose–response curves, variable temperature and radical decay at room and high annealing temperatures it was concluded that black and rooibos tea may not be a good dosimetric materials to perform accurate dose measurements in food irradiation technology. However, long-term decay data at room temperature shows that ESR technique could be used to detect (or identify) irradiated black and rooibos tea samples during the first ~10 days after the irradiation process.

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