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Food Chemistry 97 (2006) 696–704
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of γ-irradiation on antioxidant activity of black pepper (*Piper nigrum* L.)

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Received 31 January 2005; received in revised form 17 May 2005; accepted 17 May 2005

Abstract

Antioxidant activity and EPR investigations of irradiated ground black pepper (*Piper nigrum* L.) were evaluated. The black pepper was exposed to γ-irradiation at doses from 5 to 30 kGy. The effect of irradiation on antioxidant properties of black pepper extracts was investigated by radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, by determination of reducing power and content of thiobarbituric acid-reactive substances. Some significant changes were observed in creation of thiobarbituric acid-reactive substances (TBARS). Difference between non-irradiated and irradiated samples at the legal European limit dose of 10 kGy reached, on average, 23% and, at the Food and Drug Administration (FDA) 30 kGy limit, 33%. Irradiation affected significantly the DPPH radical-scavenging activity and reducing power of ground black pepper extracts. The γ-radiation treatment of ground black pepper samples observed by EPR, resulted in the production of three paramagnetic species (**GI**–**GIII**) characterized by different origin, thermal behaviour and stability. The axially symmetric EPR resonances, **GI** and **GII**, were assigned to the carbohydrate radical structures. The spin Hamiltonian parameters of **GIII** possessed the characteristic features of "cellulosic" radical species. The EPR measurements, performed 20 weeks after the radiation process, confirmed that temperature increase from 298 to 353 K, caused significant decrease of integral EPR signal intensity for γ-irradiated samples (~40%), compared to the reference (non-irradiated) ground black pepper, where only 13% drop was found. Significant correlation between EPR and thiobarbituric acid methods was assessed by study of antioxidant activity changes in relation to irradiation doses and also in the case of spice storage, between EPR and reducing power methods.

Keywords: Black pepper; γ-Irradiation; EPR spectroscopy; DPPH radical-scavenging activity; Reducing power; Thiobarbituric acid reactive substances

1. Introduction

Spice irradiation is the treatment with radiant energy to obtain some beneficial effects, which include disinfestations, improvement of the shelf life by the inactivation of spoilage organisms, and improvement of the safety of spices by inactivating food-borne pathogens. γ-Ray irradiation is now internationally recognized as an effective method for maintaining the quality of spices for a long time. The Directive 1999/3/EC established a Community list of foods and food ingredients that may be treated with ionizing radiation and maximum overall average absorbed dose may be 10 kGy for dried aromatic herbs, spices and vegetable seasonings. The FDA limit for culinary herbs, seeds, spices, vegetable seasonings, and blends of these aromatic vegetable substances is up to exceed 30 kGy (Code of Federal Regulation, 2004).

There are not many reports of the influence of irradiation procedures on antioxidant activity of herbs and spices. The effects of this processing technique on

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doi:10.1016/j.foodchem.2005.05.048

antioxidant properties of seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) were evaluated by Murcia, Egea, and Romojaro (2004). With respect to the non-irradiated samples, water extracts of irradiated spices at 1, 3, 5, and 10 kGy did not show significant differences of antioxidant activity in the radical-scavenging assays used. The antioxidant properties of anise, caraway, cumin and fennel essential oils extracted from untreated, γ-irradiated and microwaved seeds were evaluated by Farag and Khawas (1998). γ-Irradiation at 10 kGy and microwave treatments did not affect the antioxidant property of the essential oils under study. In addition, essential oils extracted from γ-irradiated fruits were more effective as an antioxidant in sunflower oil than those produced from microwaved fruits. Sun-dried and dehydrated paprika samples were irradiated at doses from 2.5 to 10 kGy and capsaicinoid contents were analysed (Topuz & Ozdemir, 2004). The contents of capsaicin, dihydrocapsaicin and homodihydrocapsaicin increased significantly by about 10% in samples irradiated at a dose of 10 kGy. The effects of irradiation, by electron beam, on the colours and on the content of volatile oils of five spice powders (prickly ash, star aniseed, cinnamon, clove and fennel) and chilli were assessed by Lianzhong, Songmei, Qiying, and Yan (1998). Irradiation enlarged the UV absorptions of aqueous extracts of spices, but darkening of spices due to irradiation was temporary. Statistical analyses of effects of irradiation on the content of volatile oils in spices showed that there were no significant differences between irradiated and non-irradiated samples. Effects of γ-irradiation, at 10 kGy, on the free radical and antioxidant contents in nine aromatic herbs and spices (basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage) were studied by Calucci, Pinzono, Zandomeneghi, and Capocchi (2003). Irradiation resulted in a general increase of quinone radical content in all of the investigated samples, as revealed by EPR spectroscopy, and in a significant decrease of total ascorbate and carotenoid content of some spices. No significant differences at 0–10 kGy were found by Calenberg et al. (1998) between EPR spectra from samples of white pepper, sweet paprika and nutmeg irradiated with electron beams or X-rays. Electron paramagnetic resonance spectroscopy was applied to study free radicals in black pepper, and to evaluate the potential of these radicals for identifying radiation treatment (Franco et al., 2004). A line produced both by radiolysis and thermolysis was observed, and its behaviour with thermal treatments suggests that it cannot be used as an irradiation indicator for doses up to 30 kGy. Two physical methods (viscosimetry with two different sample preparations and electron spin resonance) were used to detect irradiated black pepper samples (Formanek, Barabássy, Chabane, Molina, & Deyris, 1999). Identification of irradiation >8 kGy in black pepper samples is possible using these methods after one month of storage at ambient temperature. The thermal evolution behaviour of the organic free radicals induced in irradiated black pepper was studied by electron spin resonance spectroscopy (Ukai & Shimoyama, 2003a, 2003b). It was found that the radical evolution that occurred in the irradiated pepper obeyed a single exponential function and yielded a unique time constant. Powdered black pepper was irradiated with different recommended doses of γ rays (5.0 and 10.0 kGy) and with microwaves for different periods (20, 40 and 75 s) to improve its hygienic quality (Emam, Farag, & Aziz, 1995). The results obtained indicate that this treatment is a safe and suitable technique for decontamination of black pepper which does not result in a great loss of flavour compounds, as compared with recommended doses of γ-irradiation. Chabane, Pouliquen-Sonaglia, and Raffi (2001) used thermoluminescence, electron spin resonance, and viscosimetric measurements to establish whether or not a spice had been irradiated. Thermoluminescence, using the 1788 EN official protocol with an alternative method for the extraction of mineral impurities, led to proof of irradiation or proof of no treatment. Electron spin resonance led to different spectrum shapes, depending on the chemical composition of the spices. ESR could only be used as proof of irradiation up to several weeks after irradiation, and only for some spices. For identification of irradiated spices, microgel electrophoresis (DNA comet assay) may be used (Khan, Khan, & Delincée, 2002). Detection is successful in the case of poppy seeds, cardamom seeds, caraway seeds, and nigella seeds, but not in pomegranate seeds, ginger root, and juniper berries, black peppercorns, nutmeg seed, and rosemary leaves. Nevertheless, for some irradiated foods, the DNA comet assay is a rapid and inexpensive screening test. The direct epifluorescent filter technique/aerobic plate count (DEFT/APC) is the European Standard EN 13783 screening method for the detection of irradiation treatment of herbs and spices. In 1993, the European Commission gave a mandate to the European Committee for Standardisation (CEN) to standardise methods for the detection of irradiated foods. These European Standards have been adopted by the Codex Alimentarius Commission as General Methods and are referred to in the Codex General Standard for Irradiated Foods in section 6.4 on 'post-irradiation verification'. Consequently, the European Committee for Standardization (CEN) established, in EN 1787:2000, the EPR method for detection of irradiation in foods containing cellulose, and later standardized additional methods for the detection of irradiated foods, (i.e., EN 13708:2001, EN 13751:2002, and EN 13784:2001). EN 1784:1996 and EN 1785:1996 concern detection of irradiated food containing fat, and EN 1786:1996 detection of irradiated food containing bone.

Numerous studies deal with detection methods for irradiated herbs and spices and have also established that food irradiation can be considered a radiologically, microbiologically, and toxicologically safe technology. Nevertheless, questions focussing on nutrient loss, free radicals and radiolytic by-product formation, and changes of antioxidant properties during irradiation are still being debated in the scientific field. This article contributes to this topic with some results from the testing of antioxidant activity of irradiated black pepper (*Piper nigrum* L.).

2. Materials and methods

2.1. Materials and sample preparation

For the antioxidant activity and EPR investigations, a ground black pepper (density 550 g/l) from Vietnam was used. This spice was irradiated using a ^{60}Co source at doses of 5, 7.5, 10, 20, and 30 kGy according to commercial practices at Artim, s.r.o., Prague, Czech Republic on November 7, 2003. Thermal treatment of black pepper was realised by sterilization at 130 °C, by means of dry steam for 2–4.5 min (internal temperature of black pepper at sterilization was 92–98 °C) and the effect of this treatment on black pepper was studied by EPR measurement. Mean dry matter content of ground black pepper immediately after irradiation was 87.9%.

Determination of some antioxidant properties was achieved with extracts prepared from 2 g black pepper extracted for 1 h with 50 ml 80% methanol. The irradiated samples of black pepper used for antioxidant activity determinations were stored at laboratory conditions.

2.2. DPPH radical-scavenging assay

The DPPH radical-scavenging assay was modified according to Bandonienė, Murkovic, Pfanhauser, Venskutonis, and Gruzdiene (2002). A quantity of 0.65 ml of methanolic black pepper extract was added to 25 ml methanolic solution of DPPH and absorbance at 515 nm was measured after 15 min. Radical-scavenging activity was calculated as

$$\% = (\text{absorbance of control} - \text{absorbance of sample}) \times 100/\text{absorbance of control.}$$

2.3. Thiobarbituric acid number

Thiobarbituric acid-reactive substances (TBARS) were determined according to the method of Zin (2002). To 1 ml of methanolic black pepper extract, 20% aq. trichloroacetic acid (2 ml) and of aq. thiobarbituric acid solution (2 ml) were added. This mixture was then placed in a boiling water bath for 10 min. After cooling it was centrifuged at 3000 rpm for 20 min. Thiobarbituric acid number was determined as absorbance of supernatant at 532 nm.

2.4. Reducing power

Determination of reducing power was realized according to Chyau, Tsai, Ko, and Mau (2002). Black pepper methanolic extract (2 ml) was mixed with 2 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2 ml of 1% potassium ferricyanide, and the mixture was incubated at 50 °C for 20 min. After 2 ml of 10% trichloroacetic acid were added, the mixture was centrifugated at 200*g* for 10 min. The upper layer 1 ml was mixed with 1 ml distilled water and 0.2 ml of 0.1% ferric chloride, and the absorbance was read after 1 min at 700 nm in a spectrophotometer.

2.5. EPR measurements

The homogenized ground black pepper powders (100 mg) were placed in thin-wall quartz EPR tubes (internal diameter of 3 mm, length 150 mm, and wall thickness about 0.1 mm) to produce cylindrical samples with identical dimensions (sample column height 2.6 ± 0.2 cm). The sample was then inserted into a standard TE_{102} (ER 4102 ST) rectangular cavity of an EMX X-band EPR spectrometer (Bruker, Germany) and the EPR spectrum was recorded at various temperatures. Temperature control was achieved using a Bruker temperature control unit ER 4111 VT. The careful filling procedure of EPR cells resulted in good reproducibility between samples with a standard deviation in the relative EPR intensity of ±5% for five independent measurements. The EPR spectrometer settings were as follows: microwave frequency, 9.45 GHz; microwave power, 0.63–31.73 mW; centre field, 335.4 mT; sweep width, 20–500 mT; gain, 5×10^5; modulation amplitude, 0.05 mT; modulation frequency, 100 kHz; scan, 84 s; time constant, 40.96 ms, number of scans, 5; temperature, 298–373 K. The *g*-values were determined with uncertainty of ±0.0005 by simultaneous measurement of a reference sample containing DPPH fixed on EPR cell. The EPR instrument settings for quantitative evaluation were tested by the DPPH standard.

The experimental EPR spectra processing and simulation was carried out using *WIN EPR* and *SimFonia* programmes (Bruker). The integral intensities of the EPR signals were obtained by double integration of the spectrum. The multi-component experimental EPR spectra were evaluated as a linear combination of individual EPR spectra simulations using a least-squares minimization procedure with the *Scientist Program* (MicroMath). The statistical parameters of the calculation procedure (R^2, coefficient of determination and

correlation) serve as a determination of the simulation quality, i.e., correlation of the experimental and simulated spectra. The relative concentration of the individual paramagnetic species was evaluated from the contributions of the individual simulations to the experimental spectrum after double integration.

2.6. Spectrophotometric measurements

For the spectral measurements of DPPH radical-scavenging activity, thiobarbituric acid value and reducing power, the UV–Vis Specord M40 (Carl Zeiss Jena, Germany) was used under the conditions: spectral bandwidth 0 cm^{-1}, integration time 1 s, gain 3. For measurement a square cell with path length of 1 cm was used.

2.7. Statistics

For statistical purposes, ANOVA – Analysis of Variance (one factor) – was used at the significance level of 0.05. For all determinations, three replicates of sample were done.

3. Results and discussion

3.1. Antioxidant activity

Fig. 1 shows the results of DPPH-scavenging activity changes of irradiated black pepper, measured in methanolic extracts. Irradiation resulted in a significant tendency to decreasing of DPPH radical-scavenging activity of black pepper methanolic extracts, mainly immediately after irradiation and after the first month of irradiation. Found differences gradually disappeared during the storage of irradiated black pepper. In contrast to the effect of irradiation doses, very important changes in DPPH radical-scavenging activity were found during the storage of irradiated and non-irradiated black pepper. Statistically significant increase of DPPH activity was observed after two months of both, irradiated and non-irradiated black pepper storage. After the fifth month of storage, changes reached about 4–9%. This increase of black pepper radical-scavenging activity may be due to the increase of dry matter content (about 4% w/w) during the storage of this spice under laboratory conditions (Table 1). Subsequently, the anti-radical activity changes caused by storage were not so significant.

Fig. 2 shows the effect of irradiation doses and storage time on reducing power of methanolic extracts of ground black pepper. Found differences in reducing power of this spice were significant according to the irradiation doses used. Very significant changes were observed during the storage of irradiated black pepper. After the fourth month of black pepper storage, the decrease of reducing power of irradiated and non-irradiated spice extracts reached over 10%. In contrast to the previously mentioned DPPH radical-scavenging activity, i.e., increase during the storage of irradiated and non-irradiated ground black pepper, the decrease of reducing power may be caused by deactivation of some spice antioxidants reducing Fe^{3+} to Fe^{2+}. These changes are probably more intense as there is an increase of radical-scavenging substance content due to moisture changes during the storage of black pepper, as mentioned above.

Fig. 3 shows the effects of irradiation and storage of ground black pepper on changes of thiobarbituric acid number determined in methanolic extracts of black pepper. These results well represent the increase of reactive substances in black pepper caused by ionizing radiation. Increase of these substances is proportional to the dose of irradiation. Statistically significant

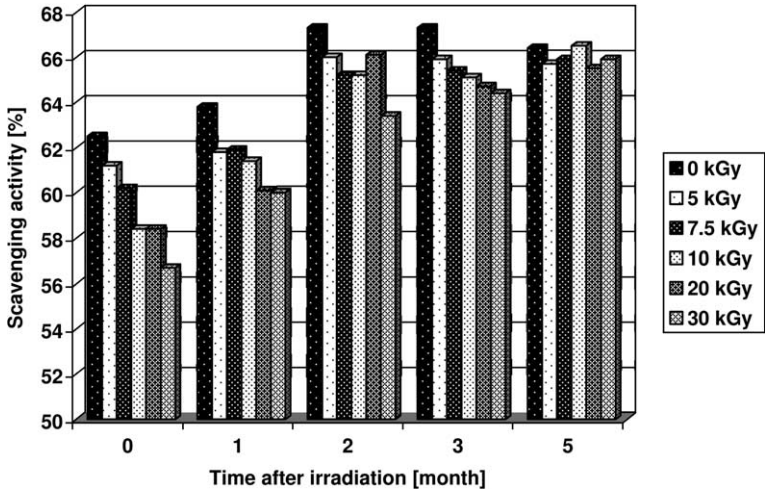

Fig. 1. Effect of irradiation and storage time on DPPH radical-scavenging activity of black pepper extracts.

Table 1
Changes of dry matter content during the storage of irradiated black pepper

Time[a] (month)	Dry matter (% w/w) of black pepper irradiated at					
	0 kGy	5 kGy	7.5 kGy	10 kGy	20 kGy	30 kGy
0	88.13	87.90	87.84	87.81	87.99	87.92
3	90.80	90.93	91.01	90.82	90.76	90.86
6	91.94	92.14	92.04	92.00	92.15	92.09

[a] Time after irradiation of black pepper stored under laboratory conditions.

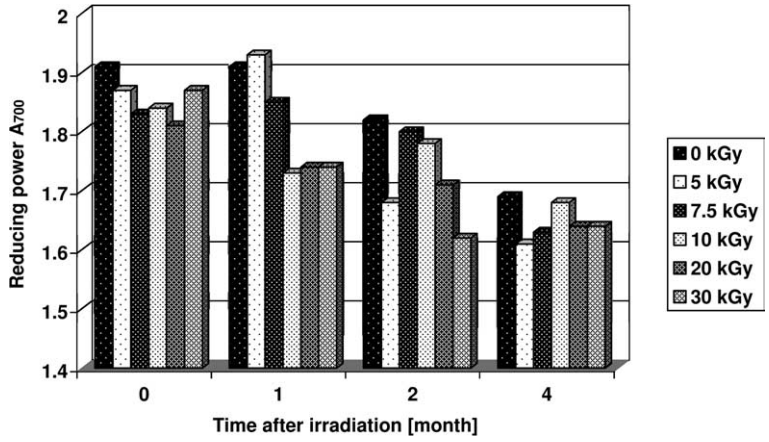

Fig. 2. Effect of irradiation and storage time on reducing power of black pepper extracts.

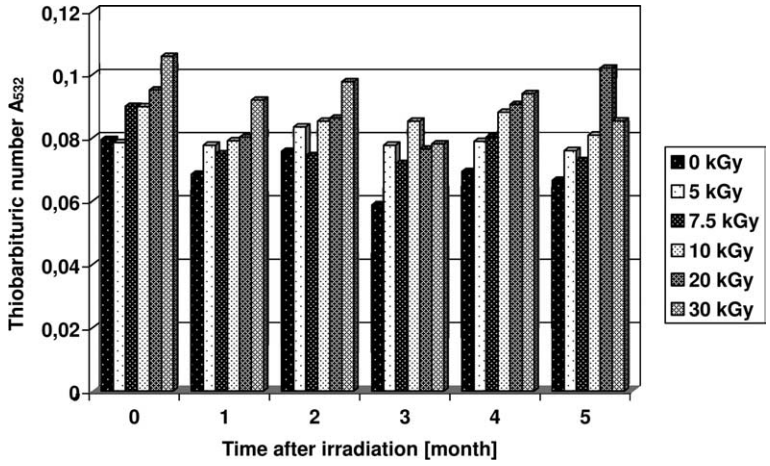

Fig. 3. Effect of irradiation and storage time on thiobarbituric number of black pepper extracts.

difference between non-irradiated and irradiated samples, at the legal European limit dose 10 kGy, reached, on average, 23% and, the FDA 30 kGy limit, up to 33%. Differences of TBARS values of extracts caused by storage of irradiated and non-irradiated ground black pepper were not significant. The effect of irradiation doses on the thiobarbituric acid values was traceable, even five months after irradiation of black pepper. The results with this antioxidant activity marker are in a quite good relation to the below mentioned EPR measurements.

3.2. EPR spectra of ground black pepper samples

Fig. 4 shows the X-band EPR spectra of the reference (non-irradiated) sample, together with samples γ-irradiated at various doses measured at 298 K one week after γ-radiation treatment. In addition, the EPR spectra of a thermally treated reference sample (labelled 0T) are also shown. The EPR spectra, recorded using a broad magnetic field sweep width (500 mT), clearly demonstrated a broad singlet line with unresolved hyperfine splittings attributed to Mn(II) ions (Lozak, Soltyk, Ostapczuk, &

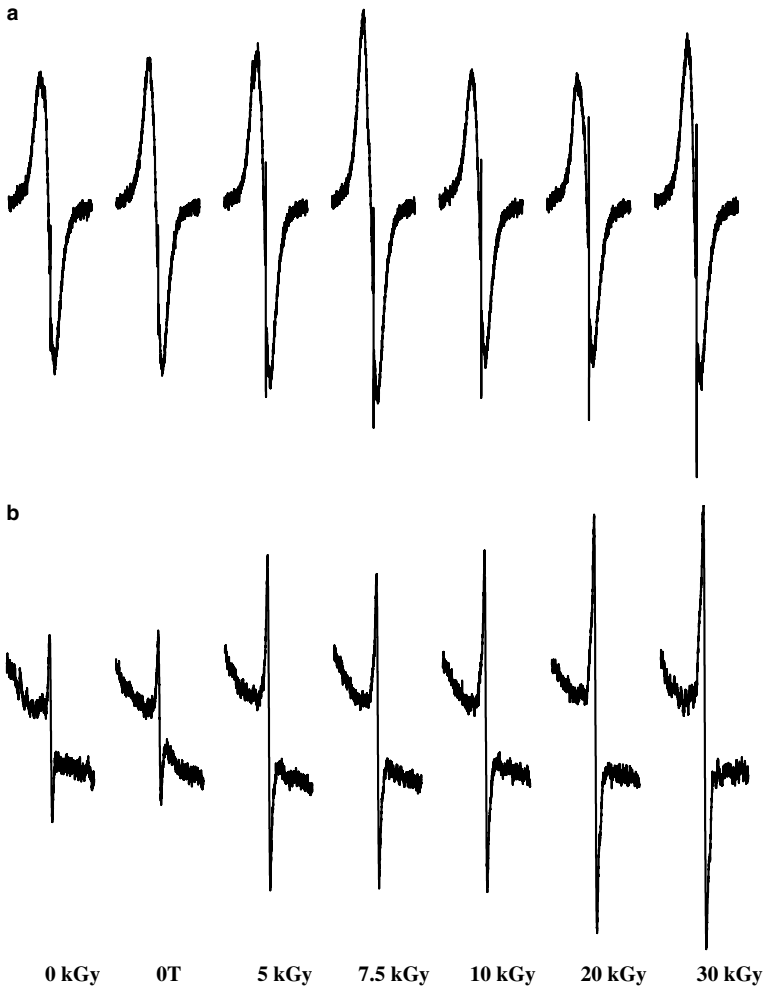

Fig. 4. X-band EPR spectra of ground black pepper samples before and after γ-radiation treatment at various doses, measured at 298 K using microwave power 0.633 mW and magnetic field sweep width: (a) 500 mT; (b) 20 mT. (0 – non-irradiated (reference) sample; 0T – thermal treatment of reference sample. EPR spectra were recorded 1 week after γ-radiation.)

Fijalek, 2002; Morsy, 2002; Morsy & Khaled, 2001, 2002; Polat & Korkmaz, 2003; Polovka, Brezová, & Staško, 2003), upon which is superimposed a sharp EPR signal, whose intensity increases with increasing γ-radiation dose (Fig. 4(a)). Fig. 4(b) shows an expanded view of the sharp EPR signals (magnetic field sweep width of 20 mT).

Simulation of the reference sample EPR spectra confirmed the presence of two individual paramagnetic resonances (**PI** and **PII**). The first EPR signal, **PI**, represents a sharp singlet line characterized by $g_{iso} = 2.0050$ and $\Delta B_{pp} = 0.55$ mT which can be attributed to semiquinone radicals produced by the oxidation of polyphenolic compounds present in plants (Jezierski et al., 2002; Morsy & Khaled, 2001, 2002; Pedersen, 2002; Polovka et al., 2003; Ukai & Shimoyama, 2003a, 2003b). The second EPR signal, **PII**, is described by an axially symmetric line ($g_{\perp} = 2.0062$, $g_{\parallel} = 2.0052$; $\Delta B_{pp} = 5.0$ mT) and can be assigned to the mechanically induced free radicals produced during the grinding process. It should be noted that the EPR spectra of the thermally treated reference samples (0T) were fully compatible with the EPR signals of **PI** and **PII**.

For comparison, sharp signals ($g_{iso} = 2.0073$) superimposed on an Mn(II) sextet in the EPR spectra of cell wall residue extracted from wheat straw were also interpreted as a semiquinone radical species produced by the one-electron oxidation of lignin phenolic groups (Merdy, Guillon, Dumonceau, & Aplincourt, 2002), and recently, the EPR spectra of untreated black pepper powder were described by a Lorentzian singlet line ($g_{iso} = 2.0044$) which was attributed to a peroxyl radical, in which the intensity increased upon heating at 373 K (Franco et al., 2004).

The EPR spectra of γ-irradiated ground black pepper samples revealed the formation of three radiation-induced EPR signals (**GI**–**GIII**) attributed to a combination of carbohydrate radical structures (**GI** and **GII**) (Korkmaz & Polat, 2001; Vanhaelewyn et al., 2000), and a typical "cellulosic" signal **GIII** (Bayram & Delincée,

2004; Kispéter et al., 2003; Yordanov & Aleksieva, 2004; Yordanov & Gancheva, 2000; Yordanov et al., 1998).

The changes in the EPR spectra of non-irradiated and γ-radiation processed black pepper samples were monitored during 20 weeks of storage in darkness (temperature 6 °C; relative humidity 60%). The storage time after radiation treatment substantially influenced only EPR spectra of γ-irradiated samples, as the EPR spectra of reference sample remained intact. However, in all γ-radiation-treated samples, an exponential decline of integral EPR intensity was observed, as is illustrated in Fig. 5(a) for samples treated at a dose of 30 kGy. The inset in Fig. 5(a) reveals the changes in EPR spectra observed 20 weeks after the γ-radiation process. The analysis of the experimental spectra shows that the decrease of EPR intensity on storage time for γ-radiation-induced signals, **GI**–**GIII**, can be described by the formal first-order kinetic model, and the evaluated half-times confirmed the lowest stability of the "cellulosic" triplet signal (Polovka et al., 2005). This information is in correlation with data published previously, which recommended the direct EPR identification of black pepper γ-radiation treatment during 6–7 weeks after the radiation process (Bayram & Delincée, 2004; Delincée, 2002; Raffi et al., 2000; Yordanov et al., 1998).

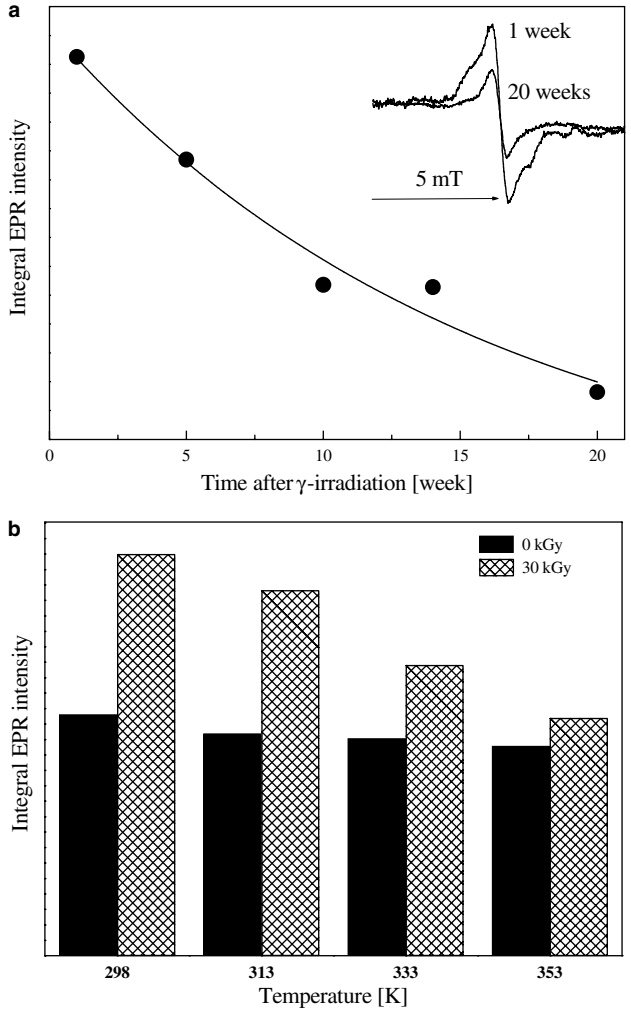

Fig. 5. (a) The dependence of relative integral EPR intensity of ground black pepper samples on storage time after γ-radiation, evaluated from EPR spectra measured at 298 K using 0.633 mW microwave power. Inset represents the experimental EPR spectra of sample γ-irradiated at dose 30 kGy monitored 1 week and 20 weeks after radiation. (b) Influence of increasing temperature on the integral EPR intensity, monitored for non-irradiated and γ-irradiated (dose 30 kGy) black pepper samples measured 20 weeks after radiation treatment.

Additionally, we compared the temperature changes of EPR spectra for non-irradiated and γ-irradiated samples 20 weeks after γ-radiation treatments as is illustrated in Fig. 5(b). The loss of integral EPR intensity in non-irradiated sample, monitored upon temperature increase from 298 to 353 K, represented 13%, while the analogous temperature change losses caused in the γ-irradiated sample (dose 30 kGy) were 40%. The results obtained are fully compatible with technique specified for identification of food γ-radiation treatments for long time periods after the radiation process (Yordanov & Gancheva, 2000).

4. Conclusions

Irradiation of ground black pepper at the doses studied, shows some significant influences on the antioxidant activities. With respect to the non-irradiated samples the most significant changes of antioxidant activity were observed in generation of thiobarbituric acid-reactive substances, where the difference at the legal European limit dose of 10 kGy reached on average, 23% and, at the FDA limit value of 30 kGy, 33%. Irradiation, at tested doses, significantly affected the DPPH radical-scavenging activity and reducing power of ground black pepper extracts.

The presented EPR investigations of γ-irradiated black pepper samples confirmed the limited life-times of three radiolytically produced paramagnetic species. The lowest stability was evaluated for the "cellulosic" EPR signal ($g_{\perp} = 2.0029$, $g_{\parallel} = 2.0014$; $A_{\perp} = 3.00$ mT, $A_{\parallel} = 1.80$ mT; half-time of 4 weeks). However, the γ-irradiated samples containing cellulose could be recognized, as well, longer times after γ-radiation treatment, due to the different consequence of the temperature increase, from 298 to 353 K, on the EPR signal intensity monitored for non-irradiated and γ-radiation-processed samples.

Significant correlation between EPR and thiobarbituric acid methods was assessed by study of antioxidant activity changes in relation to irradiation doses and, in the case of spice storage, between EPR and reducing power methods.

Acknowledgements

Authors thank the Ministry of Education of Slovak Republic (Subprogramme "Foodstuff – Quality and Safety", Registration No.: 2003SP270280E010280E01) and Slovak Grant Agency (Project VEGA/1/0053/03) for the financial support.

References

Bandonienė, D., Murkovic, M., Pfanhauser, W., Venskutonis, P. R., & Gruzdiene, D. (2002). Detection and activity evaluation of radical scavenging compounds by using DPPH free radical and on-line HPLC–DPPH methods. *European Food Research Technology, 214*, 143–147.

Bayram, G., & Delincée, H. (2004). Identification of irradiated Turkish foodstuffs combining various physical detection methods. *Food Control, 15*, 81–91.

Calenberg, S., Vanhaelewyn, G., Cleemput, O., Callens, F., Mondelaers, W., & Huyghebaert, A. (1998). Comparison of the effect of X-ray and electron beam irradiation on some selected spices. *Lebensmittel-Wissenschaft und-Technologie, 31*, 252–258.

Calucci, L., Pinzono, C., Zandomeneghi, M., & Capocchi, A. (2003). Effects of γ-irradiation on the free radical and antioxidant contents in nine aromatic herbs and spices. *Journal of Agricultural and Food Chemistry, 51*, 927–934.

Chabane, S., Pouliquen-Sonaglia, I., & Raffi, J. (2001). Detection of irradiated spices by different physical techniques. *Canadian Journal of Physiology and Pharmacology/Review of Canadian Physiology and Pharmacology, 79*, 103–108.

Chyau, C. C., Tsai, S. Y., Ko, P. T., & Mau, J. L. (2002). Antioxidant properties of solvent extracts from *Terminalia catappa* leaves. *Food Chemistry, 78*, 483–488.

Code of Federal Regulation: 21CFR179: *Irradiation in the production, processing and handling of food*, Title 21 (Vol. 3). Revised as of April 1, 2004.

Delincée, H. (2002). Analytical methods to identify irradiated food – a review. *Radiation Physics and Chemistry, 63*, 455–458.

Directive 1999/3/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of foods and food ingredients treated with ionising radiation. *Official Journal of the European Communities*, 1999, L66/24.

Emam, O. A., Farag, S. A., & Aziz, N. H. (1995). Comparative effects of gamma and microwave irradiation on the quality of black pepper. *Zeitschrift für Lebensmittel-Untersuchung-und Forschung, 201*, 57–561.

European Standard EN 13708 (2001). Detection of irradiated food containing crystalline sugar by ESR spectroscopy. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 13751 (2002). Detection of irradiated food using photostimulated luminescence. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 13783 (2001). Detection of irradiated food using Direct Epifluorescent Filter Technique/Aerobic Plate Count (DEFT/APC) – Screening method. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 13784 (2001). DNA comet assay for the detection of irradiated foodstuffs – Screening method. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 1784 (1996). Detection of irradiated food containing fat – Gas chromatographic analysis of hydrocarbons. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 1785 (1996). Detection of irradiated food containing fat – Gas chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 1786 (1996). Detection of irradiated food containing bone – Method by ESR spectroscopy. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 1787 (2000). Determination of irradiated food containing cellulose: Method by ESR spectroscopy. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

European Standard EN 1788 (2001). Thermoluminescence detection of irradiated food from which silicate minerals can be isolated – Method by thermoluminescence. (European Committee for Standardization, rue de Stassart 36, B-1050 Brussels, Belgium).

Farag, R. S., & Khawas, K. H. (1998). Influence of gamma-irradiation and microwaves on the antioxidant property of some essential oils. *International Journal of Food Sciences and Nutrition, 49*, 109–115.

Formanek, Z., Barabássy, S., Chabane, S., Molina, C., & Deyris, V. (1999). Identification of irradiation in coded black pepper samples by different physical methods (viscosimetry and ESR). *Acta Alimentaria, 28*, 103–109.

Franco, R. W. A., Neto, L. M., Kato, M. S. A., Furlan, G. R., Walder, J. M. M, & Colnago, L. A. (2004). Identification of irradiation treatment in black pepper by electron paramagnetic resonance. *International Journal of Food Science and Technology, 39*, 395–401.

Jezierski, A., Czechowski, F., Jerzykiewicz, M., Golonka, I., Drozd, J., Bylinska, E., et al. (2002). Quantitative EPR study on free radicals in the natural polyphenols interacting with metal ions and other environmental pollutants. *Spectrochimica Acta A, 58*, 1293–1300.

Khan, A. A., Khan, H. M., & Delincée, H. (2002). Identification of irradiated spices using the novel technique of DNA comet assay. *Journal of Food Science, 67*, 493–496.

Kispéter, J., Bajúsz-Kabók, K., Fekete, M., Szabó, G., Fodor, E., & Páli, T. (2003). Changes induced in spice paprika powder by treatment with ionizing radiation and saturated steam. *Radiation Physics and Chemistry, 68*, 893–900.

Korkmaz, M., & Polat, M. (2001). Radical kinetics and characterization of the free radicals in gamma-irradiated red pepper. *Radiation Physics and Chemistry, 62*, 411–424.

Lianzhong, D., Songmei, Z., Qiying, G., & Yan, Z. (1998). *Study on irradiation sterilization of spices*. Institute of Applied Technical Physics of Zhejiang Province, Hangzhou 310012, China. Available from http://epaper.kek.jp/a98/APAC98/6D059.PDF.

Lozak, A., Soltyk, K., Ostapczuk, P., & Fijalek, Z. (2002). Determination of selected trace elements in herbs and their infusions. *Science of the Total Environment, 289*, 33–40.

Merdy, P., Guillon, E., Dumonceau, J., & Aplincourt, M. (2002). Characterization of a wheat straw cell wall residue by various techniques. A comparative study with a synthetic and an extracted lignin. *Analytica Chimica Acta, 459*, 133–142.

Morsy, M. A. (2002). Teas: Direct test on quality and antioxidant activity using Electron paramagnetic resonance spectroscopy. *Spectroscopy – International Journal, 16*, 371–378.

Morsy, M. A., & Khaled, M. M. (2001). Direct electron paramagnetic resonance study of tobacco. 1. Manganese(II) as a marker. *Journal of Agricultural and Food Chemistry, 49*, 683–686.

Morsy, M. A., & Khaled, M. M. (2002). Novel EPR characterization of the antioxidant activity of tea leaves. *Spectrochimica Acta A, 58*, 1271–1277.

Murcia, M. A., Egea, I., & Romojaro, J. (2004). Antioxidant evaluation in dessert spices compared with common food additives. Influence of irradiation procedure. *Journal of Agricultural and Food Chemistry, 52*, 1872–1881.

Pedersen, J. A. (2002). On the application of electron paramagnetic resonance in the study of naturally occurring quinones and quinols. *Spectrochimica Acta A, 58*, 1257–1270.

Polat, M., & Korkmaz, M. (2003). The effect of temperature and storage time on the resonance signals of irradiated pea (*Pisum sativum* L.). *Food Research International, 8*, 857–862.

Polovka, M., Brezová, V., & Staško, A. (2003). Antioxidant properties of tea investigated by EPR spectroscopy. *Biophysical Chemistry, 106*, 39–56.

Polovka, M., Brezová, V., Staško, A., Mazúr, M., Suhaj, M., & Šimko, P. (2005). EPR investigations of gamma-irradiated ground black pepper. *Radiation Physics and Chemistry* (submitted).

Raffi, J., Yordanov, N. D., Chabane, S., Douifi, L., Gancheva, V., & Ivanova, S. (2000). Identification of irradiation treatment of aromatic herbs, spices and fruits by electron paramagnetic resonance and thermoluminescence. *Spectrochimica Acta A, 56*, 409–416.

Topuz, A., & Ozdemir, F. (2004). Influences of gamma irradiation and storage on the capsaicinoids of sun-dried and dehydrated paprika. *Food Chemistry, 86*, 509–515.

Ukai, M., & Shimoyama, Y. (2003a). An electron spin resonance study of evolution of organic free radicals in irradiated pepper during heat treatment. *Journal of Food Science, 68*(7), 2225–2229.

Ukai, M., & Shimoyama, Y. (2003b). Free radicals in irradiated pepper: an electron spin resonance study. *Applied Magnetic Resonance, 24*, 1–11.

Vanhaelewyn, G., Sadlo, J., Callens, F., Mondelaers, W., De Frenne, D., & Matthys, P. (2000). A decomposition study of the EPR spectrum of irradiated sucrose. *Applied Radiations and Isotopes, 52*, 1221–1227.

Yordanov, N. D., & Aleksieva, K. (2004). X- and Q-band EPR studies on fine powders of irradiated plants. New approach for detection of their radiation history by using Q-band EPR spectrometry. *Radiation Physics and Chemistry, 69*, 59–64.

Yordanov, N. D., & Gancheva, V. (2000). A new approach for extension of the identification period of irradiated cellulose-containing foodstuffs by EPR spectroscopy. *Applied Radiation and Isotopes, 52*, 1958-198.

Yordanov, N. D., Gancheva, V., Radicheva, M., Hristova, B., Guelev, M., & Penchev, P. (1998). Comparative identification of irradiated herbs by the methods of electron paramagnetic resonance and thermoluminescence. *Spectrochimica Acta A, 54*, 2413–2419.

Zin, Z. M. (2002). Antioxidative activity of extracts from Mengkudu (*Morina citrifolia* L.) root, fruit and leaf. *Food Chemistry, 78*, 227–231.