Study of Irradiated Black Pepper Antioxidant Activity Changes

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Abstract: Some antioxidant activities of extracts of irradiated black pepper (*Piper nigrum*) were evaluated. The ground black pepper was exposed to gamma-irradiation at doses from 5 to 30 kGy. The effect of irradiation on antioxidant properties of black pepper extracts was investigated by these methods: radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, determination of reducing power, thiobarbituric acid reactive substances assay and electron paramagnetic resonance spectroscopy (EPR). Irradiation resulted in a decrease of DPPH antiradical activity of black pepper extracts according to dose of irradiation. Reducing power was not changed by action of irradiation. Very important and sensitive differences in antioxidant activity of irradiated black pepper were investigated by EPR spectrometry and thiobarbituric acid method.

Keywords: black pepper; ionizing radiation; antioxidant activity

INTRODUCTION

Spices 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 foodborne pathogens. γ-Ray irradiation is now internationally recognized as an effective method to maintain the quality of spices for a long time. The Directive 1999/3/EC [1] 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. Not many contributions concern the influence of irradiation procedures on antioxidant activity of herbs and spices. This procedure was evaluated for seven dessert spices (anise, cinnamon, ginger, licorice, mint, nutmeg, and vanilla) [2]. Irradiated samples at 1, 3, 5, and 10 kGy did not show significant differences in the antioxidant activity with respect to the non-irradiated samples in the assays used. Nine spice and aromatic herb samples (basil, bird pepper, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, and sage) were γ-irradiated at a dose of 10 kGy according to commercial practices [3]. Irradiation resulted in a general increase of quinine radical content in all of the investigated samples, as revealed by EPR spectroscopy, and in a significant decrease of ascorbate and carotenoids. No significant differences at 0–10 kGy were found between EPR spectra from samples of white pepper, sweet paprika and nutmeg irradiated with electron beams or X-rays [4]. The antioxidant property of anise, caraway, cumin and fennel essential oils extracted from untreated, gamma-irradiated (10 kGy) and microwaved seeds was evaluated [5]. Gamma-irradiation and microwave treatments did not affect the antioxidant property of the essential oils under study. 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 pepper samples were irradiated at doses from 2.5 to 10 kGy and capsaicinoid contents were analyzed. The increases of capsaicin, dihydrocapsaicin and homodihydrocapsaicin significantly increased about 10% with the dose of 10 kGy [6]. 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 [3]. Nevertheless, questions focusing on nutrient loss, free radicals and radiolytic byproducts formation, and changes of antioxidant properties during irradiation are still being debated in the scientific field and results from antioxidant activity studies will help to increase of knowledge base in the field of irradiated food quality.

EXPERIMENTAL

For the study of antioxidant activity changes a ground black pepper (density 550) from Vietnam was used. This spice was irradiated at doses of 5, 7.5, 10, 20, and 30 kGy according to commercial practices (Artim, s. r. o., Prague, Czech Republic). Determination of some antioxidant properties was made with extracts prepared from 2 g black pepper extracted for 1 h with 50 ml 80% methanol.

DPPH radical scavenging assay was modified according to [7]. 0.65 ml of methanolic black pepper extract was placed into 25 ml methanolic solution of DPPH and absorbance at 515 nm was measured after 15 min. Radical scavenging activity was calculated as % = (absorbance of control – absorbance of sample) × 100/absorbance of control.

Thiobarbituric acid number was determined according to method [8].To 1 ml of sample extract, 20% aq. of 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 an absorbance of supernatant at 532 nm.

Reducing power was realized according to method [9]. Sample extract (2 ml) was mixed with 2 ml of 0.2M 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 was added, the mixture was centrifugated at 200 × g 10 min. 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 spectrophotometer.

For EPR spectrum measurement of irradiated black pepper a Spektrometer EMX EPR (Bruker, Germany) was used. Samples were measured in a solid phase in 3 mm cuvette, volume of sample 100 mg [10]. For the others spectral measurements UV-VIS Specord M40 (Carl Zeiss Jena, Germany) was used.

RESULTS AND DISCUSSION

Figure 1 shows the results of DPPH scavenging activity changes of irradiated black pepper measured in methanolic extracts during the 5 month. The most differences in DPPH scavenging activities among the used doses were investigated immediately after black pepper irradiation. At a dose of 30 kGy difference in antiradical activity between irradiated and non irradiated black pepper samples was 5.8%, and 4.1% at a legal European limit dose 10 kGy. Some months after irradiation of black pepper antiradical activities of extracts were approximately identical.

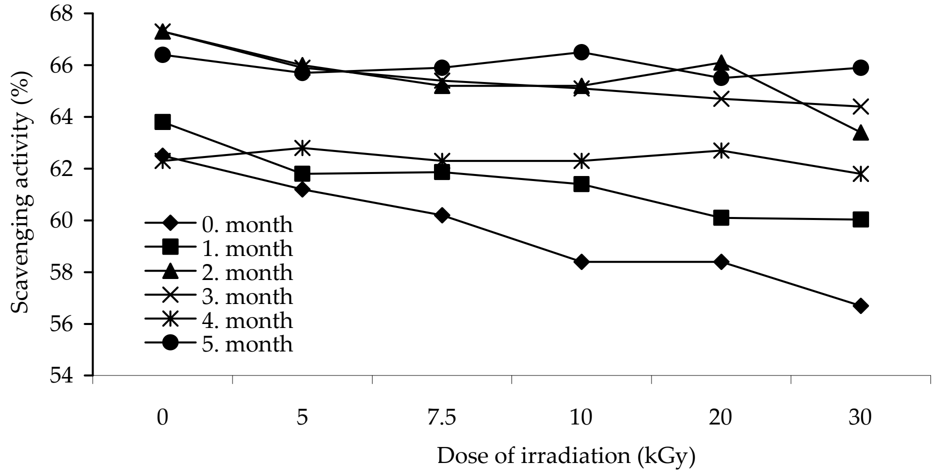

Figure 1. DPPH scavenging activity changes of irradiated black pepper extracts

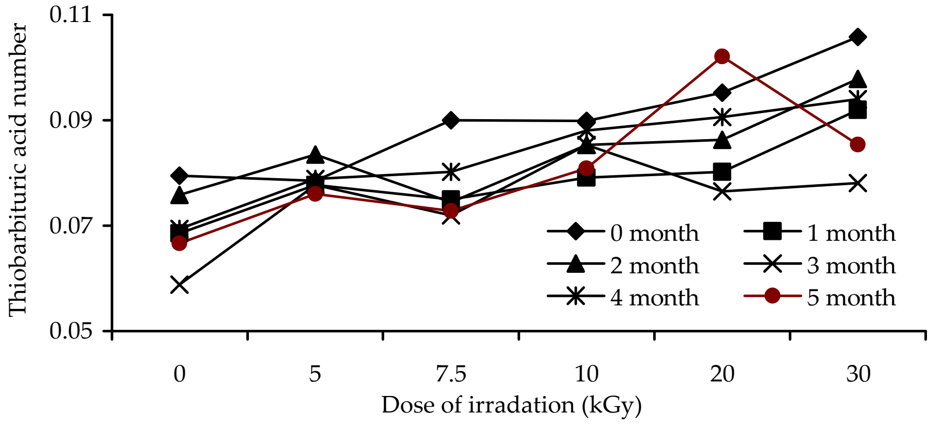

Figure 2. Thiobarbituric acid reactive substances in irradiated black pepper extracts

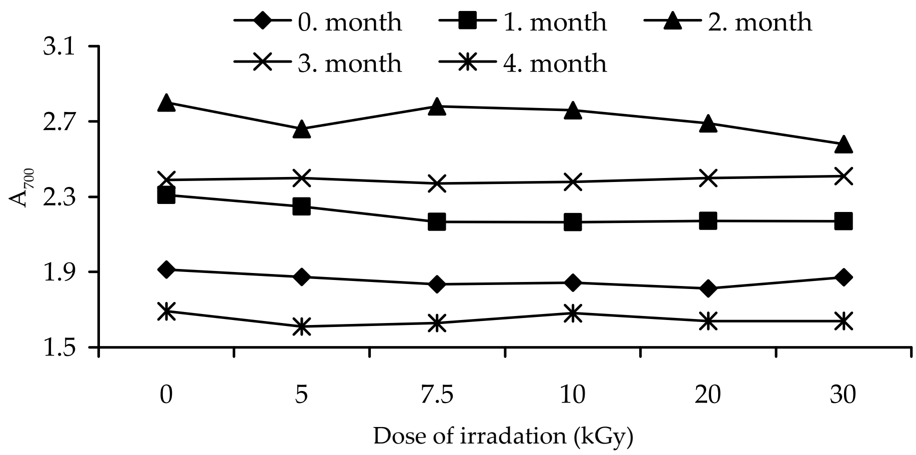

Figure 3. Reducing power of irradiated black pepper extracts

Figure 2 shows the results of thiobarbituric acid number determined in irradiated black pepper extracts. These results well represent the increase of reactive substances in black pepper by subsequence of ionizing radiation. Increase of these substances is proportional to the dose of irradiation. Differences between non irradiated and irradiated sample at 30 kGy were approximately 30% in each of observed month and at a legal European limit dose about 20%.

Differences in reducing power of methanolic extracts of irradiated black pepper (Figure 3) were not significant

Very important and sensitive differences in antioxidant activity of irradiated black pepper were investigated by EPR spectrometry [10]. Radiation causes creation of two new paramagnetic signals. With the increasing of irradiation dose gradually increases of relative intensity of EPR signal (Figure 4) [10].

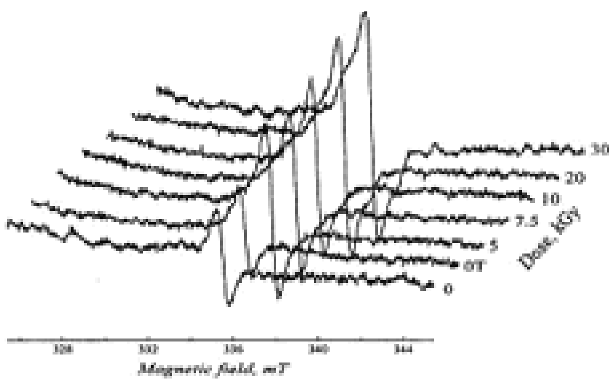

Figure 4. EPR spectrum of irradiated black pepper [10]

CONCLUSIONS

Irradiation of ground black pepper at the doses studied shows some significant influences on the antioxidant activities. Some significant changes were observed at DPPH antiradical scavenging activity, in creation of thiobarbituric acid reactive substances (TBARS) and increasing of EPR signal. The changes in DPPH activity and TBARS assay can be explained by increasing of yield from extraction of irradiated black pepper as well. Radiation can induce disruption of the cell wall structure and consequent higher extractability [2].

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