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Effect of absorbed dose and storage length on electron paramagnetic resonance (EPR) signal strength in irradiated alfalfa seeds

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Abstract A kind of alfalfa seeds was irradiated by 1, 2, 3, 4 and 5 kGy at a dose rate of 6.288 kGy·h^{-1} in a self-shielded irradiator of ^{137}Cs gamma rays. The EPR spectra, which were measured subsequently between 0.3401 and 0.3501 T, showed that there was a direct proportional relationship between the EPR signal strength of free radicals produced by gamma irradiation in the alfalfa seeds and absorbed dose. The first derivative EPR spectra of the alfalfa seeds were very clear and easy to identify. However, the EPR signal strength of the peak-to-peak amplitude decreased rapidly and most of them decayed beyond 50% within 3 days after the seeds were irradiated. It tended to stabilize after half a month since the seeds were irradiated. The differences of the EPR signal strength between the irradiated and unirradiated alfalfa seeds still remained. All seeds were stored at ambient temperature for more than 3 months. Therefore, using EPR spectrometry technique to measure free radicals in alfalfa seeds as a means to determine whether the seeds have been irradiated or not is feasible, relatively fast and simple.

Key words Alfalfa seeds, EPR spectrum, Free radicals, Gamma irradiation, Detection

CLC numbers S124$^+$.1, O657.2

1 Introduction

Human salmonellosis associated with the consumption of alfalfa seed sprouts has been documented in several countries and people have applied several techniques to kill microorganisms in the alfalfa seeds[1]. Gamma irradiation can effectively kill the microorganisms in the seeds over a certain range of absorbed dose[2]. As a prerequisite, a suitable detection method for distinguishing whether or not a kind of food has been irradiated lies not only in whether some differences between unirradiated and irradiated food can be found, but also in whether the differences can continuously keep on a certain stable phase. Therefore, a relatively fast, simple and sensitive technique to distinguish between irradiated and unirradiated alfalfa seeds is required. The free radicals produced by irradiation in the alfalfa seeds can be detected by electron paramagnetic resonance (EPR) spectrometry technique[3]—one of the most advanced analytical methods for detecting radiation-processed foods. The aim of this experiment was to study the effect of absorbed doses and storage length on the EPR signal strength of free radicals produced by irradiation in the alfalfa seeds so as to confirm whether the EPR spectrometry techniques is a suitable detection method for irradiated alfalfa seeds.

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2 Materials and methods

A kind of alfalfa seeds, which comes from Caudill seed, has been used in this experiment. The samples of the alfalfa seeds were placed in small glass bottles, and divided into six groups. Five groups of them were irradiated by 1, 2, 3, 4 and 5 kGy, respectively, at an absorbed dose rate of 6.288 $kGy \cdot h^{-1}$. The remaining group was used as a control. Samples were irradiated using a ^{137}Cs self-shielded irradiator with an elevator sample chamber. The dose rate of the chamber was calibrated by an alanine EPR dosimetry system (E 1607-94, ASTM Standards)[4], which can be traced to national standard at National Institute of Standards and Technology (NIST). The dose is reported as the absorbed dose in water. Corrections for the different mass absorption coefficients for ionizing photon irradiation of the seeds are not necessary.[5] The temperature in the chamber was controlled at about 20°C by a temperature controller of liquid nitrogen. Seeds were measured immediately using an EMS 104 EPR analyzer after they were irradiated. The EPR signal intensities were monitored continuously. Sample was placed in quartz EPR tube with a diameter of 0.32 cm, in which the sample of seeds weighed about 100 mg. The optimization of experimental parameters in the EPR spectrometer for this experiment was taken. Calibration for the spectrometer instrument with the standard sample was carried out at the beginning of each measurement so that each measurement of the sample can be taken at the same instrument condition. To ensure uniformity and reproducibility of the sample measurements condition all alfalfa seed samples were kept inside the EPR tubes after the first measurement, and each time they were measured subsequently at the same fixed position with the same sample height in the EPR cavity. All samples in the EPR tubes were stored and measured at room temperature.

3 Results and discussion

Table 1 showed that absorbed dose could directly influence the EPR signal strength of free radicals produced by irradiation of alfalfa seeds. The EPR signal strength of samples increased linearly along with the absorbed dose (Fig. 1). The result that the absorbed dose of the alfalfa seeds can be estimated this way is significant. However, the irradiation-induced EPR signal strength of free radicals in the alfalfa seeds was not stable. The degradation rates of the irradiation-induced EPR signal strength presented a direct proportional relationship with the absorbed dose of the alfalfa seeds during several days after the seeds were irradiated. The decrease of the irradiation-induced EPR signal strength on the samples, whose absorbed dose was larger than 2 kGy in this experiment was all beyond 50% of the original signal strength (Fig. 2). Although initial decay rates were nearly linear, the decay rates tended to be subsiding, fluctuating and relatively stabilizing with time. The decay of irradiation-induced EPR signal strength gradually tended to placidity and stability after the EPR signal strength decayed rapidly. The average fluctuation of the EPR signal strengths on the five groups of samples was ± 1.26 from half a month after the samples had been irradiated (control was ± 1.11) (Table 2).

Table 1 Effect of absorbed dose on EPR signal strength in irradiated alfalfa seeds

Absorbed dose / kGy	Signal strength (% maximum)
0	6
1	34
2	54
3	74
4	108
5	126

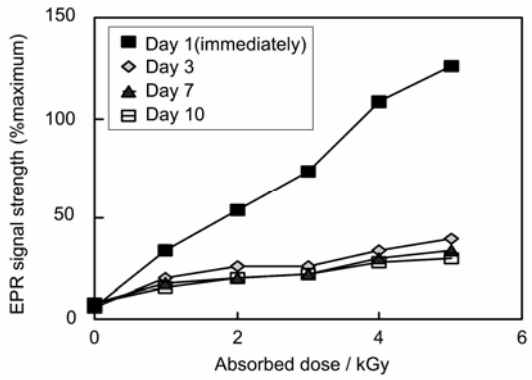

Fig.1 Effect of absorbed dose and storage length on the EPR signal strength in irradiated alfalfa seeds.

Table 2 Effect of storage length on EPR signal strength in irradiated alfalfa seeds (%maximum signal strength)

Measurement No.	Storage length / d	Absorbed dose / kGy					
		0	1	2	3	4	5
1	0	6	34	54	74	108	126
2	3	6	20	26	26	34	40
3	7	8	18	20	22	30	34
4	10	8	16	20	22	28	30
5	14	6	14	18	20	24	28
6	22	8	14	18	20	24	28
7	25	8	16	16	20	24	28
8	29	8	16	16	20	24	26
9	35	8	14	16	18	22	26
10	42	8	14	14	18	22	24
11	49	8	14	16	18	22	26
12	57	8	14	16	18	22	26
13	63	8	14	14	18	22	24
14	72	8	14	14	18	22	24
15	78	10	14	14	18	22	24
16	92	10	14	14	16	22	22
17	105	10	14	14	18	22	24
S^*_{n-1}		1.1094	0.7511	1.5021	1.198	0.96	1.89

* No. 5—17.

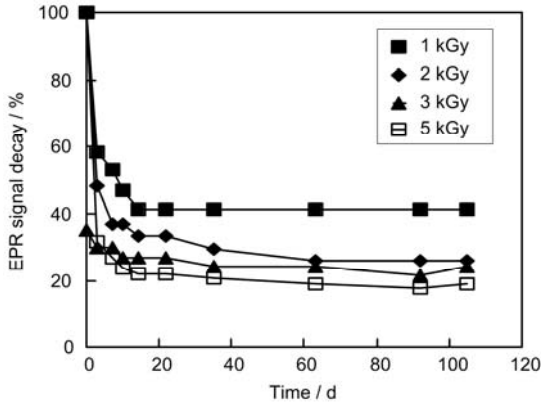

Fig.2 Time-dependent change in radiation-induced EPR signal strength for alfalfa seeds expressed as percent change in the signal measured immediately after irradiation.

The irradiation-induced EPR signal strength of the five groups of samples are kept higher than that of the control from beginning to end in this experiment (Fig. 3), which means that using EPR spectrometry technique to measure EPR signal strength of free radicals produced by irradiation in the alfalfa seeds is an effective, fast and simple method to distinguish whether or not the seeds have been irradiated. However, the facts whether different kinds of alfalfa seeds have the same EPR spectra and signal strength, what should be the "threshold value", and which is the minimum absorbed dose so that the irradiated alfalfa seeds can be distinguished are still unknown. Since no studies have been conducted so far for them, to the best of our knowledge, further experiments are required.

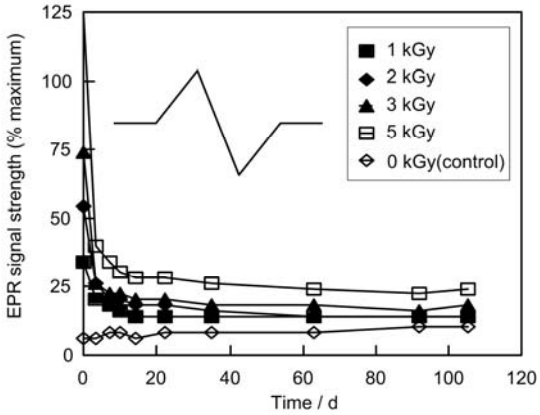

Fig.3 Effect of storage length on EPR signal strength for irradiated alfalfa seeds ($g = 2.0036 \pm 0.0002$).

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