Radiation Physics and Chemistry 117 (2015) 41–47

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Evaluation of absorbed dose in irradiated sugar-containing plant material (peony roots) by an ESR method

Rumi Yamaoki [a,*], Shojiro Kimura [a], Masatoshi Ohta [b]

[a] Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094, Japan
[b] Faculty of Engineering, Niigata University, 8050 Igarashi 2-Nocho, Nishi-ku, Niigata 950-2181, Japan

HIGHLIGHTS

- Irradiated peony roots revealed a sugar-like ESR spectrum.
- The radical concentration had nearly stabilized 30 days after irradiation.
- The ESR spectrum was composed of overlapping of radical signals from carbohydrates.
- The ESR signal intensity of sucrose radical was increased proportionally up to 20 kGy.
- The ESR signal intensity of sucrose radical was correlated to the sucrose content.

ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form
29 June 2015
Accepted 19 July 2015
Available online 20 July 2015

Keywords:
ESR
Irradiation
Radical concentration
Sugar content
Sucrose
Peony root

ABSTRACT

The relationship between electron spin resonance (ESR) signal intensity of irradiated plant materials and sugar content was investigated by spectral analysis using peony roots.

A weak background signal near $g=2.005$ was observed in the roots. After a 10 kGy irradiation, the ESR line broadened and the intensity increased, and the spectral characteristics were similar to a typical spectrum of irradiated food containing crystalline sugars. The free radical concentration was nearly stable 30 days after irradiation. The spectrum of peony root 30 days after irradiation was simulated using the summation of the intensities of six assumed components: radical signals derived from (a) sucrose, (b) glucose, (c) fructose, (d) cellulose, (e) the background signal near $g=2.005$ and (f) unidentified component. The simulated spectra using the six components were in agreement with the observed sample spectra. The intensity of sucrose radical signal in irradiated samples increased proportionally up to 20 kGy. In addition, the intensity of sucrose radical signals was strongly correlated with the sucrose contents of the samples. The results showed that the radiation sensitivity of sucrose in peony roots was influenced little by other plant constituents. There was also a good correlation between the total area of the spectra and the sucrose content, because the sucrose content was higher than that of other sugars in the samples. In peony roots, estimation of the absorbed dose from the ESR signal intensity may be possible by a calibration method based on the sucrose content.

1. Introduction

Irradiation of foods and drugs is an effective technique for disinfecting and sterilizing raw materials. Ionizing radiation from gamma rays, electron beams, and X-rays produces free radicals and ions in the constituents of the materials and destroys adhering microorganisms, either by direct effects on DNA or through production of radicals and ions that attack DNA. Most radicals are short-lived because of their high reactivity. Some radicals are trapped in substances and are relatively stable. Electron spin resonance (ESR) is highly sensitive for detecting radicals induced by irradiation and allows speedy analysis without complex preparation of samples. In the European Union and Japan, ESR analysis is approved as one of the standard reference methods for the detection of irradiated food containing bone, cellulose and crystalline sugar (EN1786, 1996; EN1787, 2000; EN13708, 2001; Notice no. 0910 article 2 of the Department of Food Safety, 2012).

Organic radicals derived from crystalline sugars and cellulose are known to be long-lived compared to other radicals derived from plant constituents. In particular, radicals from crystalline

* Corresponding author. Fax: +81 726 90 1020.
E-mail address: yamaoki@gly.oups.ac.jp (R. Yamaoki).

http://dx.doi.org/10.1016/j.radphyschem.2015.07.010
0969-806X/

monosaccharides and disaccharides have been reported to be highly stable (Yamauchi et al., 1999; Yordanov and Georgieva, 2004). Sugar radicals produce asymmetrically divided ESR signals with an overall spectrum width of more than 6 mT (center of spectrum near $g=2.004$). The sugar spectrum is very durable and observation of the spectrum is very useful for detection of irradiated dried papayas, raisins, dried mangoes and dried figs (Raffi, 1992; Raffi et al., 1992; Helle et al., 1992). However, the detection limit and the stability are influenced by the crystallinity and quantity of sugar. Few attempts have been made to identify the specific radicals responsible for individual signals or to relate the amounts of plant constituents to the ESR signal intensity (Raffi and Agnel, 1989; Mischke et al., 1994). One reason is that ESR spectra are complex overlapping signals due to multiple organic radicals derived from plant tissues. If the possible spectral components of irradiated plant materials can be isolated and analysis focused on more stable radical species, ESR is expected to become a more quantitative detection method. Furthermore, dose determination using stable radical species would lead to a strong guarantee of a reduction in number of microorganisms on the irradiated materials.

Peony root, an herbal medicine, is used as a remedy in various diseases of women, and its characteristic constituents are astragalin, benzoic acid, calcium, copper, gallic acid, glucose, linoleic acid, magnesium, paeoniflorin, paeonol, potassium, tannin, zinc, and sucrose (Natural Resources Conservation Service, 2014). ESR spectral characteristics similar to those of irradiated food containing crystalline sugars have been observed in irradiated samples (Yamaoki et al., 2007). In this study, the major ESR components of irradiated peony root were identified by spectral analysis, and the relationship between the ESR signal intensity and the sugar content was investigated. Estimation of the absorbed dose using sucrose radicals generated in irradiated plants was evaluated.

2. Materials and methods

2.1. Materials

Dried roots of *Paeonia lactiflora* (Paeoniae Radix, peony root) were used as plant materials. Nine peony roots (cut into small pieces) from North Korea and China were obtained from wholesalers. Crystalline monosaccharides and disaccharides (fructose, glucose, and sucrose) were obtained from Nacalai Tesque. Cellulose powder was obtained from Sigma-Aldrich. Samples were stored at room temperature in the dark (22 ± 2 °C, 50 ± 10% RH). Samples were dried at 105 °C for 5 h and weighed accurately to constant mass to determine the % loss on drying (The Japanese Pharmacopoeia Fifteenth Edition, 2005).

2.2. Analysis of monosaccharides and disaccharides

A 3 g aliquot of each sample was added to 15 ml warm water and sonicated for 5 min. The solution was made up to 25 ml with H_2O and centrifuged at 3000 rpm for 5 min. A 5.0 ml aliquot of the solution was diluted to a volume of 10 ml with ethanol and filtered through a 0.45 μm membrane filter. Separation and detection were carried out using a Shimadzu high performance liquid chromatography (HPLC) system (C-VP series) with injection volume 6 μl; an NH_2P-504E column (Asahipak, 4.6 mm i.d., 250 mm), 30 °C; a mobile phase of acetonitrile–water (75:25); flow rate 1 ml/min; detection with a differential refractometer (Yasui et al., 1996). Calibration curves were prepared with standard solutions using analytical grade fructose, glucose and sucrose, and the amount of each compound read using the absolute calibration curve method. Measurements were performed two or three times and the mean values were calculated (the relative error was less than 5%).

2.3. Irradiation

Samples were sealed in polyethylene bags and irradiated using a Dynamitron electron beam (EB) accelerator, 5 MeV (Radiation Dynamics, Japan Electron Beam Irradiation Service). EB irradiation (1, 5, 10, 20 kGy) was performed in room-temperature air. The absorbed dose was measured using a cellulose triacetate dosimeter (Fuji Photo Film) and a radiachromic dosimeter (Far West Technology). The dose was calculated from the difference in absorbance before and after EB irradiation, and mean values were calculated.

2.4. ESR measurement

Plant samples were ground to <2 mm. Approximately 0.1 g of sample was placed in a quartz ESR tube (5 mm). The tube was then sealed with a plastic paraffin film. ESR measurements were performed using an X-band ESR spectrometer with a 100 kHz magnetic field modulator (ES-10, Nikkiso). Measurements were at room temperature under the following conditions: microwave power, 4 mW; sweep width, 15 mT; modulation width, 0.2 mT; sweep time, 60 s; time constant, 0.12 s. The g factor was calculated from the signals of the Mn^{2+}/MgO marker. ESR signal intensity was calculated from the area of the double-integrated curve of the signal. Measurements were performed twice, and the mean values were calculated (relative error 1–5%) and corrected based on Mn^{2+}/MgO signal intensity. The radical concentration was calculated from the area of the double-integrated curve of the ESR spectrum, using 1,1-diphenyl-2-picrylhydrazyl (in benzene) as the standard.

3. Results and discussion

3.1. Water and sugar contents of peony roots

Irradiation of plants produces many free radicals. Sugar radicals and cellulose radical become the major ESR spectral components in irradiated plant materials because they are long-lived. Therefore, the ESR signal intensity is predicted to be influenced by the sugar and cellulose contents. Water content is also an important factor influencing the concentration of radicals produced by radiation. The loss of weight on drying of nine peony roots ranged from 8.54% to 14.0% in Table 1. Based on HPLC analysis of samples, the sugar content varies widely; as shown in Fig. 1, the fructose content ranged from 3.4 to 31 mg/g dry weight and the glucose content ranged from 2.6 to 33 mg/g dry weight. The sucrose content ranged from 35 to 223 mg/g dry weight, about two-fold to sixteen-fold higher than the fructose or glucose content. There was

Table 1
Water content of peony roots.

Sample no.	Loss on drying (%)
1	10.49
2	10.78
3	14.04
4	10.78
5	8.92
6	10.62
7	8.54
8	9.21
9	9.31