Electron spin relaxation behaviors of Agaricus radicals induced by irradiation

Hiromi Kameyaa,*, Mitsuko Ukaib, Hideo Nakamurab, Yuhei Shimoyamac, a Soft Matter Physics Laboratory, Graduate School of Emergent Functional Science, Muroran Institute of Technology, Muroran 050-8585, Japan.
bDepartment of Chemistry, Hokkaido University of Education, Hakodate 040-8567, Japan.
cCREST-JST, Muroran 050-8585, Japan.

*Corresponding author. fax:+81-138-43-0591.
E-mail address: kameya@science-edu.org, kimekimekimeru@yahoo.co.jp (H. Kameya)

Abstract

Agaricus (Agaricus Blazei Murill) is a popular crude drug in Japan and far east countries. The crude drugs originating from natural raw products are easy to be contaminated by microorganisms. Radiation has long been used as a sterilization technology for crude drugs. Using Electron Spin Resonance (ESR), we detect radical species in agaricus before and after the gamma ray sterilization. We determined relaxation times of radicals in agaricus in the presence and the absence of oxygen. $T_1$ values of the sample in the argon (Ar) atmosphere are two times larger than that in the air (oxygen). $T_2$ values are kept constant with and without oxygen. Knowledge on the relaxation times allows establishing a reliable protocol of ESR characterization for irradiated drugs. The ESR detection in the Ar atmosphere allows revealing the radical formation in the irradiated agaricus.

Keywords: Agaricus; ESR; $\gamma$-ray irradiation; Oxygen effects
1. Introduction

Agaricus (*Agaricus Blazei* Murill) is a common name of the agaricaceous mushroom of Brazilian origin. Agaricus is considered to be one of the crude drugs in East Asian countries. The crude drug must be sterilized because of their contamination by microorganism. Sterilization treatment on the crude drugs should be performed without spoiling drug effects. Thus, the heat-treatment cannot be used for it. Irradiation may sterilize foods and drugs effectively (Farkas, 1985).

Electron spin resonance (ESR) spectroscopy method has been applied on the detection of radicals in irradiated foods (Dodd et al, 1988). Irradiation induced radicals are detectable in the quick and exact manner by ESR spectroscopy.

In the present study, we reveal the radiation induced free radicals in agaricus for the first time by ESR spectroscopy. Thereby, we can determine the free radicals of crude drugs by comparison with these observed in non-irradiated sample. Then we unveil the spin relaxation behaviors of agaricus before and after irradiation. Such knowledge on the relaxation behaviors allows the most accurate ESR protocol of the irradiated crude drug. Further attempts to establish the oxygen effects of the ESR spectra of agaricus were made.

2. Experimental

2.1. Sample

The specimen was commercially available agaricus (Dorency, Japan) that was stored at +4 °C.
The sample was located in a quartz sample tube (99.9 % purity: Eiko, Japan) for the ESR measurements. We prepared sample tubes both in the air (open tube) and in the argon (Ar) atmosphere (sealed tube).

2.2.  Irradiation by γ-ray

The γ-ray irradiation processing was carried out at Japan Atomic Energy Agency (JAEA), Takasaki Advanced Radiation Research Institute. The radiation source was $^{60}$Co. The dose rate was 5 kGy per hour and the dose level of the γ-ray was controlled by the irradiation period. The irradiation dose levels were 0.5, 1, 2, 3, 5, 10, 30, and 50 kGy at +25 °C. The irradiation was performed in sealed tubes with Ar. Some of them were then opened before ESR measurements.

2.3.  ESR measurements

We measured ESR signal of agaricus samples by X-band CW-ESR (JEOL KK, JES-FE1XG) (Nakamura et al, 2006). We varied the incident microwave power from 0.1 to 100 mW to record the progressive saturation curves. For the calibration of the microwave power, we used the perturbing metal sphere method, in which the shift in the resonance frequency of cavity was measured by the insertion of an iron sphere of 3-mm in diameter. We found that the effective microwave powers were linear dependent on the incident powers (Shimoyama et al, 1985).
3. Results

3.1. ESR spectral features

Fig. 1 shows a typical ESR spectrum of agaricus. It consisted of three components. The sharp intense signal at $g = 2.002$ ($P_1$) was due to the organic free radical. $P_2$ signal in the vicinity of $g = 2.002$ can be assigned the sextet lines of hyperfine interaction due to Mn$^{2+}$. The $P_2$ signal indicated an asymmetric pattern whose line widths were broadened at the higher magnetic fields. To evaluate the hyperfine-coupling (hfc) constant of Mn$^{2+}$ in the agaricus samples, we used a method by taking the summit of each peak. This method yielded an identical hfc of ca. 7.4 mT. Furthermore, the signal ($P_3$) detected at $g = 4.0$ may be originated from Fe$^{3+}$ ion.

Fig. 2 shows ESR spectra of agaricus samples as recorded in the air and Ar atmosphere. These two signals are essentially the same. Peak-to-peak intensity of the agaricus in the Ar atmosphere was clearer and higher than that in the air.

The spin concentration of the organic free radical appeared at $g = 2.002$ in the irradiated agaricus was determined by the standard TEMPOL solution sample. Upon double integration, we determined the radical concentrations to be $3.8 \times 10^{14}$ and $3.4 \times 10^{16}$ spins/g for agaricus before and after irradiation, respectively. These values are two times larger than those of Ginseng (Hamaya et al, 2003). The G-value of organic radicals generation in γ-irradiated samples was evaluated to be ca. 1.0.

3.2. Progressive saturation behaviors
Fig. 3 demonstrates variations of the peak-to-peak intensity of $P_1$ at various microwave powers. Peak-to-peak intensities increased with the microwave power until the threshold point. Both $P_1$ signal from sample in the air and in the Ar atmosphere saturated at 64 and 49 mW, respectively. The progressive saturation curves indicated that the sample in the Ar atmosphere saturated at the lower microwave power than that in the air. The maximum value of $P_1$, the threshold value of sample in Ar atmosphere was higher than that in the air. Fig. 3 presents saturation behaviors of non-irradiated samples. We also measured the irradiated samples. The situation for irradiated and non-irradiated was the same.

3.3. Relaxation times

We estimated the spin lattice relaxation times ($T_1$) and spin-spin relaxation times ($T_2$) by using the Poole’s method [16]. $T_1$ and $T_2$ are given Eq. (1) and (2), respectively, in terms of $\Delta H^0 pp$ the linewidth well below saturation, $\gamma$ gyromagnetic ratio, $S$ saturation factor and $H_1$ microwave field.

$$T_1 = \frac{\sqrt{3} \Delta H^0 pp}{2 \gamma} \left( \frac{1}{S} - 1 \right)$$  \hspace{1cm} (1)

$$T_2 = \frac{2}{\sqrt{3} \gamma \Delta H^0 pp}$$  \hspace{1cm} (2)

Table 1 shows the relaxation times of sample irradiated in Ar and measured in the air and in the Ar atmosphere. We found $T_1$ and $T_2$ in $\mu$sec and nsec order, respectively. The $T_1$ value for sample in the Ar atmosphere was found to be two times larger than that in the air. However, equations above cannot be
used for inhomogeneous broadened spectra and also the Gaussian line shapes. Due to our limitation of CW-ESR instrumentation, the present method is the only way to determine $T_1$ and $T_2$, so that we decided to employ it.

3.4. Irradiation effects

Fig. 4 shows ESR spectra of agaricus (300 mg) irradiated (D=1kGy) by $\gamma$-ray and measured in the air and in the Ar atmosphere. The ESR signals were essentially the same for the three spectral lines and their $g$-values. The intensity of the $P_1$ signal increased with the irradiation dose.

4. Discussion

4.1. ESR evidences of multicomponent radical species in agaricus

We found three component signals in the X-band ESR spectrum of agaricus before irradiation. First, we found a signal at $g = 2.002$ that is due to an organic free radical. Second, we observed an intense sextet of hyperfine lines centered at $g = 2.002$ that are due to the Mn$^{2+}$ ion. The evaluated hfc constant was ca. 7.4 mT. Third, we found a signal at $g = 4.0$ due to Fe$^{3+}$ ion.

ESR evidences of multicomponent radical species in agaricus were essentially the same in ginseng radix (Nakamura et al, 2004). $P_1$ signal of ginseng was more intense than that of agaricus. For this reason, we recorded ESR signal of agaricus both in the air and in the Ar atmosphere.
4.2. Effects of radiation

Intensity of the $P_1$ signal increased remarkably according to the irradiation doses. This means that ESR method can be used for the detection of the radiation treatment. From the progressive saturation behavior, the $P_1$ signal is identified as single radical specie. The radicals of the cellulose origin have already been reported in herb and spice (Yordanov et al, 2000, Delincee et al, 2002). In case of irradiated ginseng radix, another crude drug, we have detected the corresponding twin peaks that appeared in the vicinity of the organic free radical located at $g = 2.002$ (Ukai et al, 2003, Ukai et al, 2003) In the agaricus, however, no such twin peaks was detected. This is due to the cellulose content in agaricus is less than that in ginseng radix.

4.3. Progressive saturation behavior of agaricus

The progressive saturation behaviors indicate that agaricus in Ar atmosphere saturated at the lower microwave power than that in the air. The threshold value of agaricus in Ar atmosphere occurs in the lower microwave field than that in the air. This means that $P_1$ radical is influenced by air, i.e., paramagnetic oxygen.

4.5. Influence of oxygen to the relaxation times

ESR detection in the absence of oxygen is important to observe the signal accurately, because oxygen molecule, the paramagnetic molecule leads to shorten the relaxation times. Table 1 indicated
that spin-spin relaxation ($T_2$) values were about the same with and without oxygen. On the other hand, spin-lattice relaxation ($T_1$) value of sample in the air was two times less than that in the Ar atmosphere.

$T_1$ relates to the characteristic lifetime of the spin state and is determined by the dissipation energy via the thermal vibration of the lattice. $T_2$ induces the mutual spin flips caused by dipolar and/or exchange interactions between the spins in the sample. $T_2$ is usually much shorter than $T_1$ and thus has the dominant contribution to the line width.

References


Hamaya N., Ukai M., Shimoyama Y. (2003), Thermal decay process of organic free radicals in $\gamma$-ray irradiated pepper as studied by electron spin resonance spectroscopy. Radioisotopes. 52, 367-373.


Ukai M., Shimoyama Y. (2003), Free radicals in irradiated pepper an electron spin resonance study.


Table 1

Relaxation times \((T_1 \text{ and } T_2)\) of radicals induced in the irradiated agaricus at by various atmospheric conditions. Deviation for \(T_1\) and \(T_2\) were \(\pm 0.3 \ \mu\text{sec}\) and \(\pm 0.3 \ \text{nsec}\), respectively.

<table>
<thead>
<tr>
<th>atmosphere</th>
<th>(T_1 (\mu\text{sec}))</th>
<th>(T_2 (\text{nsec}))</th>
</tr>
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<tbody>
<tr>
<td>Air</td>
<td>5.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Ar</td>
<td>9.5</td>
<td>2.1</td>
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Figure captions

Fig. 1
ESR spectrum of agaricus.

Fig. 2
ESR spectra of non-irradiated agaricus sample recorded in the air and that in the Ar atmosphere. AG means the amplifier gain.

Fig. 3
Progressive saturation behavior of the P₁ signal from agaricus before irradiation. Sample (a) is in the air, sample (b) in the Ar atmosphere. Both P₁ signal from sample in the air (a) and in the Ar (b) atmosphere saturated at 64 and 49 mW, respectively.

Fig. 4
ESR spectra of agaricus irradiated at Ar atmosphere, and recorded sample in the air and that in the Ar atmosphere. AG means the amplifier gain.
Fig. 1  ESR spectrum of agaricus.
Fig. 2 ESR spectra of irradiated agaricus sample in the air and that in the Ar atmosphere. AG means the amplifier gain.
Fig. 3 Progressive saturation behavior of the P₁ signal from agaricus before irradiation. Sample (a) was measured in the air, sample (b) - in the Ar atmosphere. Both P₁ signal from sample in the air (a) and in the Ar (b) atmosphere saturated at 64 and 49 mW, respectively.
Fig. 4 ESR spectra of agaricus irradiated at Ar atmosphere, and recorded in the air and that in the Ar atmosphere. AG means the amplifier gain.