

Electron Spin Resonance Studies of Free Radicals in γ -Irradiated Soybean Paste

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Free radicals in γ -irradiated soybean paste were investigated by electron spin resonance (ESR) spectroscopy to determine the effect of temperature (77–296 K) and moisture content (1–54%) of samples irradiated at high dose (1–40 kGy). The samples were kept in liquid nitrogen (77 K) during irradiation and subsequent ESR measurements. The spectra shown at 77 K consisted of the hydrogen atom lines at low and high field and complicated symmetric spectrum. By increasing the microwave power, the line shape of ESR spectra altered, which indicated the detection of different paramagnetic centers at different microwave powers. In saturation curves, it was possible to select four types of spectra components which were different in their relaxation times. By the different irradiation doses, the change in free radical concentration showed a curvilinearly increasing relationship with irradiation dose in wet samples, whereas a proportional relationship was observed with dried samples. This might indicate that the indirect process of free radical formation was involved with the existence of free water radicals in the wet samples.

Keywords: Free radicals; γ -irradiation; soybean paste; ESR

INTRODUCTION

The measurements of free radical concentration and its composition are important for the understanding of chemical reactions in food and biological systems, as well as for the understanding of physiological phenomena including the aging process (1–4). The difficulty of the measurement arises from the unstable (even nanosecond lifetime) nature of free radicals in food systems (5). The spin trap method and spin labeling techniques have been applied to capture free radicals in reaction (6–7). However, the dynamic of free radical formation in food systems is less understood and the methodology is not properly established.

The free radical nature may be more clearly understood if the irradiation treatment is done at low temperature, usually that of liquid nitrogen (8). In this case the radical transformation can be slow, and it may be possible to investigate the transformation by heating the sample after irradiating it at low temperature.

We have studied the radiation of fermented soybean products to improve the microbial and sensory quality of the products as raw ingredients for instant soup and condiments production (9).

On the basis of the previous results of irradiated soybeans (10–15), we have investigated ESR spectra of fermented soybean paste irradiated at 77 K to determine the effect of temperature (77–296 K) and moisture content (1–54%) on the free radical composition of samples irradiated at high doses (1–40 kGy). These

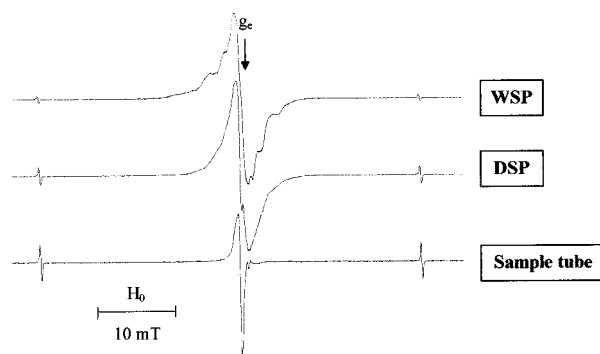


Figure 1. ESR spectra of wet soybean paste (WSP), dried soybean paste (DSP), and sample tube irradiated to 40 kGy at 77 K. The spectra were recorded at 77 K and microwave power of 5 mW.

observations could illustrate specifically the effect of enzymatic hydrolysis of soybean components by fermentation on the free radical formation during irradiation. The influences of irradiation dose, moisture content, and heating after irradiation on the free radical concentration and species of fermented soybean were examined.

MATERIALS AND METHODS

Sample Preparation. A traditional Korean fermented soybean paste, *chongkukjang*, was obtained from Pulmuone Co. in Seoul. It was made by growing *Bacillus subtilis* on cooked soybean for 2 days and mashed by adding salt. The approximate chemical composition was water (54.0%), protein (15.7%), lipid (9.0%), carbohydrate (16.1%), and ash (5.2%). For dry soybean paste (DSP), wet soybean paste (WSP) was freeze-dried, ground by a hammer mill to pass through 60 mesh screen, and stored at -20°C . To eliminate oxygen from the dry soybean paste, the sample tube was evacuated with 1×10^{-5} Torr by a vacuum pumping system (CT-2P, KVP Co., Korea) for 30 min. and then sealed by an acetylene torch.

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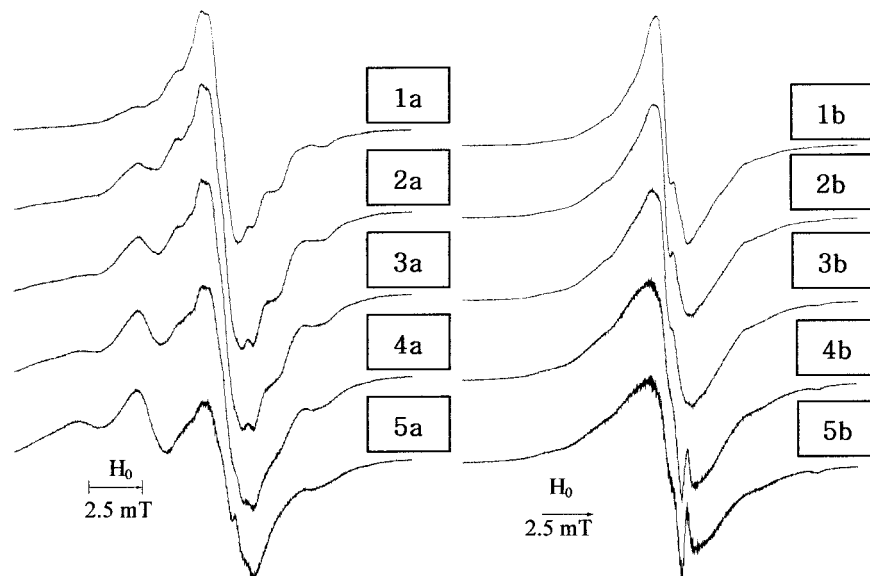


Figure 2. Central part of ESR spectra of wet soybean paste (WSP, spectrum a) and dry soybean paste (DSP, spectrum b) γ -irradiated at 77 K with the dose 40 kGy by different microwave powers; spectrum 1, 1 mW; spectrum 2, 10 mW; spectrum 3, 20 mW; spectrum 4, 100 mW; and spectrum 5, 200 mW. (Spectra recorded at 77 K.)

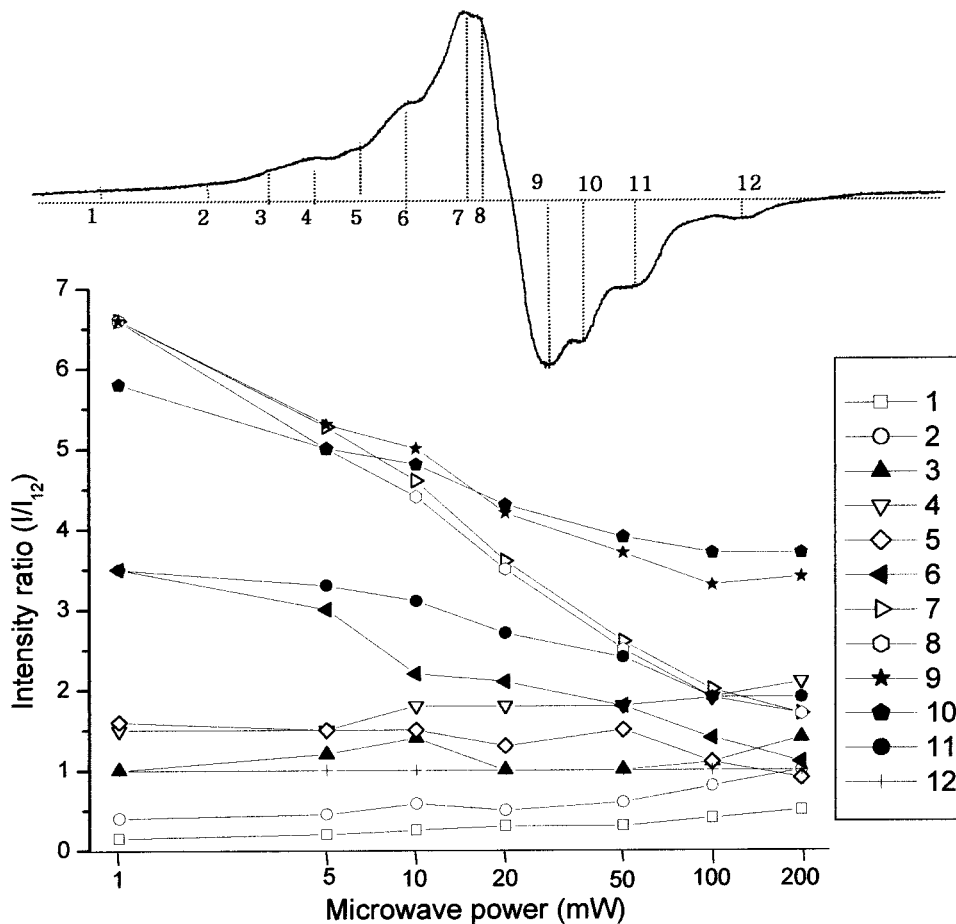


Figure 3. Saturation curves for different components of ESR spectra of wet soybean paste (WSP) γ -irradiated to 40 kGy and measured by different microwave powers. (The temperatures of γ -irradiation and ESR spectra recording were 77 K.)

Irradiation Conditions. Samples were put into ESR sample tubes (clear fused quartz, Wilmad Co., 707-SQ-250 m, Buena, NJ) and kept in liquid nitrogen throughout the irradiation process and collection of ESR measurements. Gamma irradiation was carried out with a cobalt-60 irradiator equipped with 100 kCi activity and operated at a dose rate of 7.8 kGy/hr. The absorbed dose was monitored with a ceric cerous dosimeter, and the actual dose levels, 0, 1, 2.5, 5, 7.5,

10, 15, 20, 25, 30, 35 and 40 kGy, were achieved by considering the shielding effect (30%) of the liquid nitrogen container. Because the ESR sample tube also was irradiated and an ESR signal was induced in the quartz, we irradiated the control ESR sample tube at the same conditions in order to separate the sample signals from those of the ESR sample tube.

Electron Spin Resonance (ESR) Measurements. ESR spectra were recorded on a Bruker ESP300S X band spec-

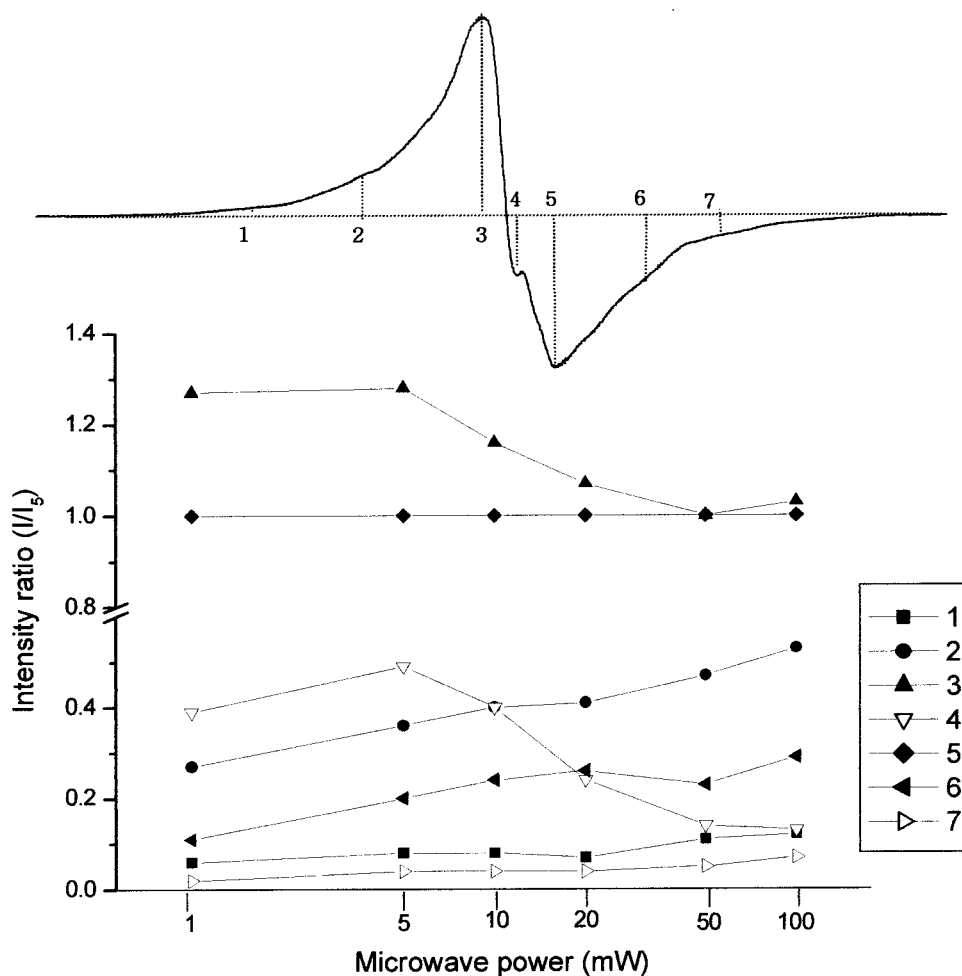


Figure 4. Saturation curves for different components of ESR spectra of dry soybean paste (DSP) γ -irradiated to 40 kGy and measured by different microwave powers. (The temperatures of γ -irradiation and ESR spectra recording were 77 K.)

trometer (Germany) at 77 K and equipped with a rectangular parallel-piped (TE102 mode) microwave cavity. The microwave frequency was 9.28 GHz. Microwave power varied from 1 mW (unsaturated ESR signal condition) to 200 mW. To investigate the thermo evolution of radicals, samples were heated to 120, 150, 190, 210, and 296 K for 5 min, and then cooled to liquid nitrogen temperature for ESR measurement.

RESULTS AND DISCUSSION

The ESR spectra of wet soybean paste irradiated to 40 kGy (WSP-40) and dry soybean paste powder irradiated to 40 kGy (DSP-40) at 77 K are shown in Figure 1. The spectra consisted of the hydrogen (H) atom lines at low and high field. The main spectra of WSP-40 and DSP-40 were near free electron position. WSP-40 showed multiplet structure, whereas DSP-40 showed mainly a singlet line with very low intensity of multiplet lines. Although the sample tube spectrum showed the doublet line of H atoms and some doublet lines near free electron position at the bottom, it was possible to propose that the H atom also stabilized in samples because the ratios of central and hydrogen signal intensities in samples were significantly higher than that of the sample tube.

Figure 2 shows the spectra at different microwave power from 1 to 200 mW. The line shape of spectra changed by microwave power, meaning that these spectra consist of different radicals. Unpaired electrons of these radicals possess different spin-spin (T_2) and spin-lattice (T_1) relaxation times. Alternated magnetic

field amplitude, H_1 is determined by microwave power, which is proportional to H_1^2 . For this reason, the relative ratio of line intensities (I) will be changed by the microwave power according to eq 1 (16):

$$I \sim H_1^2 \chi_0 \omega \omega_0 [T_2/1 + T_2^2(\omega_0 - \omega)^2 + \gamma^2 H_1^2 T_1 T_2] \quad (1)$$

where χ_0 = magnetic susceptibility, ω = microwave frequency, ω_0 = resonance microwave frequency, H_1 = magnetic field amplitude, and γ = electron gyro magnetic ratio.

The saturation curves are shown in Figures 3 and 4. The line positions shown in the figures are those indicated on spectra 1a and 1b in Figure 2. As shown in Figures 3 and 4, it was possible to select four types of spectra components which differed in their relaxation times: group I (lines 1, 2, 4, and 12); group II (lines 6 and 11); group III (lines 7 and 8); and group IV (lines 9 and 10) in WSP, and group I (lines 1 and 7); group II (lines 2 and 6); group III (lines 4 and 3); and group IV (line 5) in DSP. The different groups may indicate that they belong to the different types of radicals. The relaxation times increased as $T_1(\text{I}) < T_1(\text{II}) = T_1(\text{IV}) < T_1(\text{III})$ in WSP and $T_1(\text{I}) < T_1(\text{II}) < T_1(\text{IV}) < T_1(\text{III})$ in DSP. It shows that lines 6 and 11, and 9 and 10 in WSP may belong to the same type radicals.

Figure 5 shows the spectra obtained with 1 mW (panel a) and 200 mW (panel b) of microwave power. In wet paste (WSP-40), the $\cdot\text{OH}$ radical was properly observed

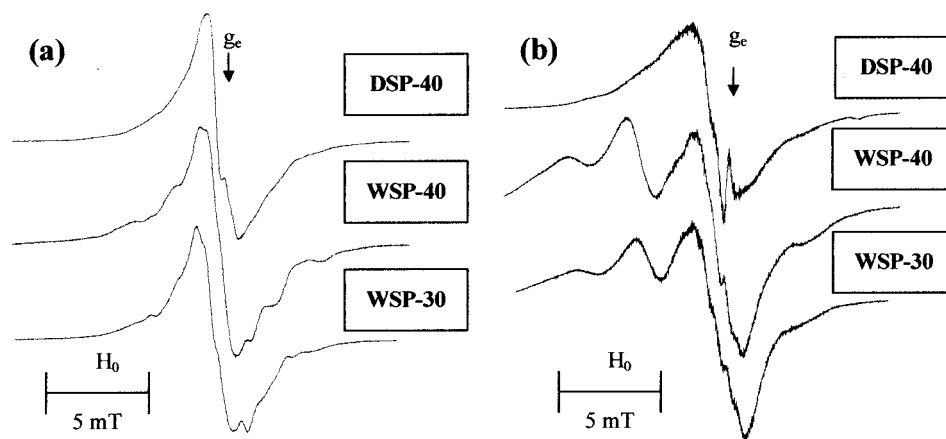


Figure 5. ESR spectra of samples measured at different microwave powers: (a), 1 mW; and (b), 200 mW. (WSP-40, wet soybean paste irradiated to 40 kGy at 77 K; WSP-30, wet soybean paste irradiated to 30 kGy at 77 K; and DSP-40, dried soybean paste irradiated to 40 kGy at 77 K). The spectra were recorded at 77 K.

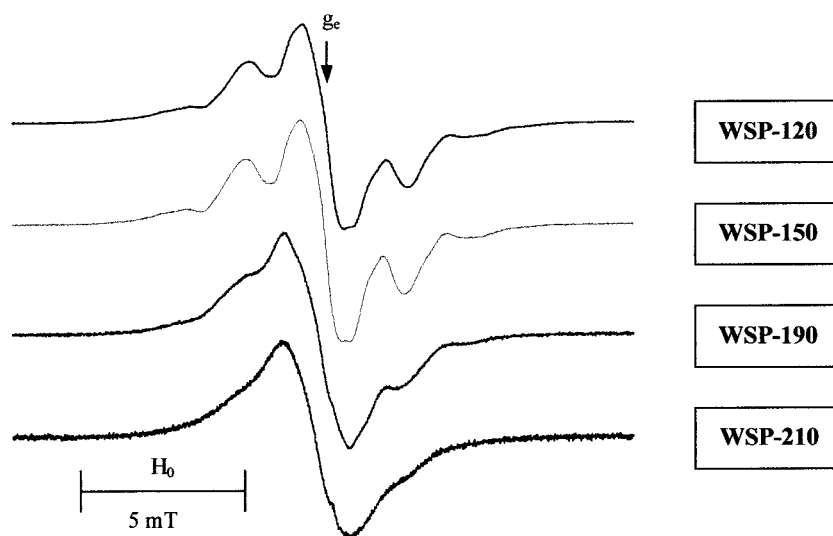


Figure 6. ESR spectra of the samples exposed at different temperatures for 5 min after irradiation to 40 kGy at 77 K. (WSP-120, wet soybean paste heated to 120 K after irradiation; WSP-150, wet soybean paste heated to 150 K after irradiation; WSP-190, wet soybean paste heated to 190 K after irradiation; and WSP-210, wet soybean paste heated to 210 K after irradiation). Microwave power was 5 mW.

with 200 mW of microwave power, because this radical has short spin relaxation time when compared to that of other paramagnetic centers, which were determined by the spectrum at low microwave power. In the dry sample (DSP-40) the water radical ($\cdot\text{OH}$) spectrum was absent. Among the multiplet lines, WSP-30 showed clearly the doublet line near the free electron position with the splitting about 2 mT that usually obtained in irradiated amino acids (17).

By heating after irradiation the $\cdot\text{OH}$ radicals disappeared and the multiplet line shape was symmetrical as shown in Figure 6. By the variation of microwave power we could see that ESR spectra are determined by three types of radicals which have different relaxation times. The relative intensities of three lines: singlet, triplet, and eight component lines, changed independently with increasing microwave power and temperature variation. Triplet and octet lines were symmetrical near $g = 2.005$ which is typical for the radicals in which the unpaired electron is localized on carbon (16). It is very important to know what part of soybean paste these lines belong to. At the elevated temperature above 0 °C some stable radicals remained

in dry sample, whereas they disappeared completely in wet samples (18).

To understand the influence of oxygen and water on the radical processes, the DSP evacuated at 1×10^{-5} Torr was tested. As shown in Figure 7, the ESR spectra of dry soybean paste (DSP) in air and in a vacuum were identical at 77 K, demonstrating that the induced radicals during irradiation were the same. By heating, however, the ways of radical recombination were different from each other. At 120 K, the ESR spectra of DSP in air and in a vacuum were similar, but the intensity of the central line in DSP in air was less than DSP in vacuum. The change in ESR line shape due to elevated temperature was aggravated by the existence of oxygen. The free radical concentration in DSP in a vacuum was not only decreased, but also transformed.

The number of paramagnetic centers in a sample may be calculated according to eq 2 (16):

$$N = \alpha[S(T - T_c) V / [\omega_0 g Q_0 P_0^{1/2} A \eta]] \quad (2)$$

where α = numerical coefficient ($\alpha = 3k/\pi^2\beta h S(S + 1)$); S = area under the absorption curve; T = temperature

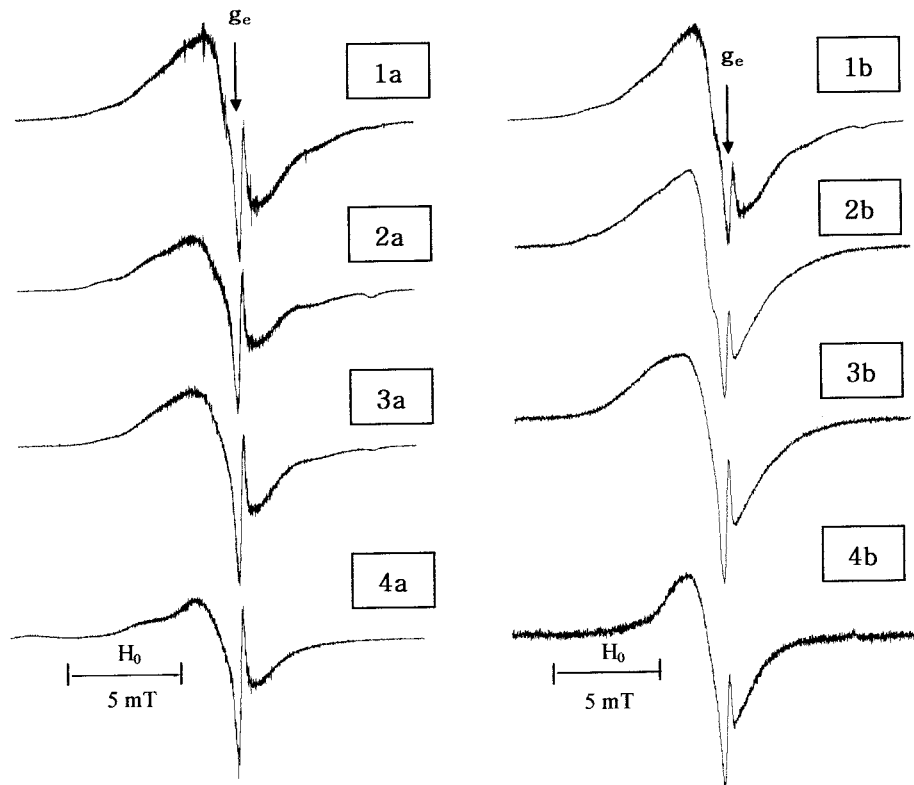


Figure 7. ESR spectra of dry soybean paste in a vacuum (a) and in air (b) measured with high (200 mW) microwave power. Samples were kept at 77 K (1), 150 K (2), 190 K (3), and 296 K (4) for 5 min respectively after irradiation at 77 K. The spectra were recorded at 77 K.

of the sample; T_c = curie temperature; V = volume of the sample; ω_0 = the resonance absorption frequency in a uniform magnetic field; Q_0 = quality factor of the resonator; A = saturation factor; P_0 = power of the klystron; and $A\eta$ = filling factor of the resonator.

However, in practice this is impossible to calculate because of the inaccurate determination of a whole series of parameters. The radical concentration is usually measured by comparing the intensities of the ESR signals of the substance being studied with a standard with a known number of paramagnetic particles (N_{st}). The paramagnetic particles number (N) and radical concentration (C) of sample were calculated from the approximate formula shown in eqs 3 and 4 (16).

$$N = N_{st} B (S/S_{st}) \quad (3)$$

$$C = N/\text{weight of sample (g)} \quad (4)$$

where $B = [Q_0^s t g^s (T - T_c) A_s t \eta V] / [Q_0 g (T - T_c)_{st} A V_{st} \eta] = 1$.

The areas S and S_{st} were found by double integration when the first derivative of the absorption signal was recorded. We used 0.005 g of copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) single crystal for standard material. When the molecular weight of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was 250 g, the molecule number was Avogadro's number, 6×10^{23} . Therefore the molecule number (N_{st}) of 0.005 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was calculated to 1.2×10^{19} and the signal area (S_{st}) of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was 2.055×10^9 . For example, when the signal area (S) of 1 g of WSP at 5 kGy was 9.11×10^8 , the number of paramagnetic particles (N), and thus the radical concentration (C), was calculated to 5.32×10^{18} .

Figure 8 shows the free radical concentrations calculated by means of double integration of the ESR

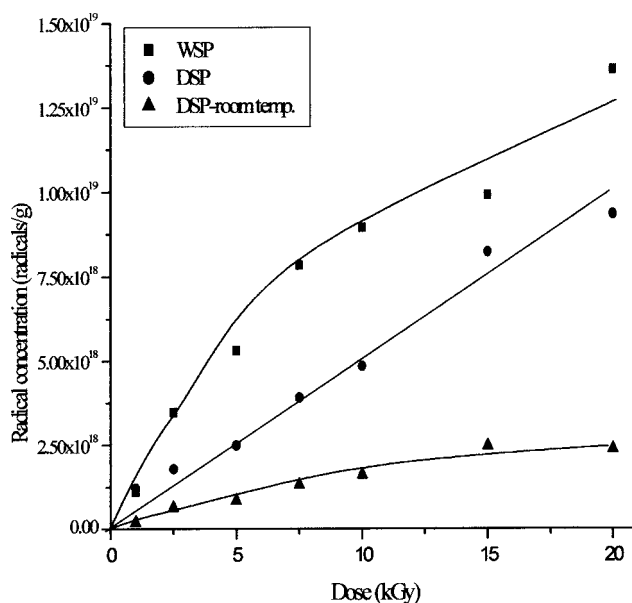


Figure 8. Dose dependence of average radical concentration of fermented soybean paste products irradiated and recorded at 77 K. (WSP, wet soybean paste; DSP, dried soybean paste; and DSP-room temp., dried soybean paste irradiated at 77 K and then exposed to room temperature for 15 min after irradiation). The spectra were recorded at 77 K.

spectrum of wet soybean paste (WSP), dry soybean paste (DSP), and DSP exposed to room temperature for 15 min after irradiation.

The free radical concentration in WSP was much higher than that of DSP at the lower irradiation doses. In wet sample, the changes in free radical concentration showed curvilinear relation with irradiation dose; the free radical concentrations increased rapidly by the

irradiation to 1–5 kGy, but slowly by the high dose. On the other hand, the free radical concentration in the dry sample was much lower than that of the wet sample and showed a proportional relationship with irradiation dose. This may indicate that the indirect process of free radical formation was involved with the existence of free water radicals in the wet sample (19). A large number of free radicals disappeared by heating to room temperature for 15 min. So we could expect direct process in dry samples and indirect processes in wet samples to occur.

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