# Electron Spin Resonance Analysis of $\gamma$-induced Free Radicals in Riceberry Rice Grain

**P Rintarak[1,5], B Pattanasiri[2], S Anupong[3], S Ninlaphruk[4] and S Dangtip[1,5*]**

[1]Dept of Physics & NANOTEC CoE, Faculty of Science, Mahidol University, Bangkok, Thailand
[2]Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, Thailand
[3]Dept of Chemistry, Faculty of Science, Mahidol University, Bangkok, Thailand
[4]Office of Atoms for Peace, Bangkok, Thailand
[5]ThEP Center, Commission for Higher Education, Bangkok, Thailand

E-mail: somsak.dan@mahidol.edu

**Abstract.** Gamma irradiation has long been serving as one alternative preservation method for food and agriculture products. Initial physical interactions of gamma with biomaterials lead through radical formation, depolymerization, and molecular changes to eventual sterilization, decontamination and preservation. An effective penetration of gamma radiation homogenize the effects throughout the materials. Riceberry rice composes mainly of starch, which has $\alpha$-D glucopyranose ($C_6H_{12}O_6$ or glucose) as a building block in its polysaccharide network. Follow irradiating rice with Co-60 gammas of a few kilograys, the polysaccharide chains have undergone several changes in their molecular structure, which lead to generation of free radicals. Electron spin resonance (ESR) analysis has shown that quite a few free radicals were formed with different strengths. Their respective Landes g-values were in the range of 2.0020 to 2.0039. Mechanisms such as hydrogen abstraction on C1 and C6 position and formation of primary hydroxyalkyl between C1 and C6 are presumably accounted for the formation of these free radicals. However, the unknown species which is singlet shape was found in this study.

## 1. Introduction

Nowadays, the irradiation plays an important preventive role such as to reduce humidity, to suppress germination, and to lower insect contamination to storage goods or products in their long logistics. The most common $\gamma$-ray irradiation sources are from Co-60. Riceberry rice has an essential nutrition and is becoming popular for health-concern consumers in Thailand. The effects of $\gamma$-ray irradiation on the particular rice cultivars, riceberry, has not been studied in details. Some previous studies of gamma irradiation on rice have reported that $\gamma$-ray irradiation can promote radical formation. [1–6]. Such a process may be understood by following an action series from primary physical interactions such as photoelectric and Compton effects to radical formations. However, each interactions in the series may vary in strength, in time, and may subject to other competing processes of different degree, etc., are thus complicates. As such, the promising concept is to follow interactions of $\gamma$-ray irradiation right with the basic building block of carbohydrate such as glucose (figure 1) [2–4]. A group of researchers has focused their work to radiolysis on glucose as a source of radical creations [3, 4]. On the other hand, other research groups have focused their attention to the generation of free electrons by the photoelectric and Compton interactions of $\gamma$ with glucose as main source of radical formation [6, 7].

Electron spin resonance (ESR) has been a common technique to study of free radicals formation from radiolysis [4]. The ESR spectrum indicated radical exhibit and can separate different feature between the irradiated rice and the native rice. Anyway, some works reported that the native rice cannot exhibit ESR [2]. The spectral parameters from ESR technique which are Landé g-value and hyperfine coupling constant are related to origin of radical formation. Reconstruction by using the spectral parameters is simulated by EasySpin and Orca software. Therefore, the goal in this study is to verify free-radicals structure in riceberry rice group by using ESR spectroscopy and to discuss radical formation from $\gamma$-D glucopyanose with which ESR spectrum exhibit.

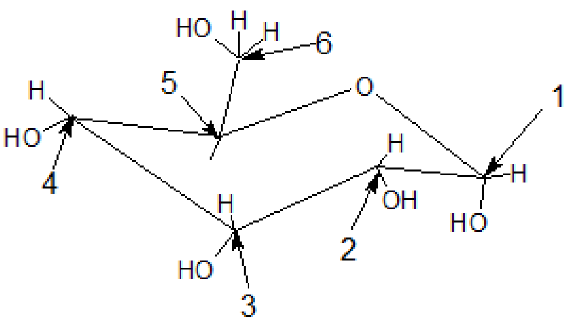


**Figure 1.** Molecular structure of $\alpha$-D glucopyranose or glucose. Arrows with number indicates position of carbon atoms .

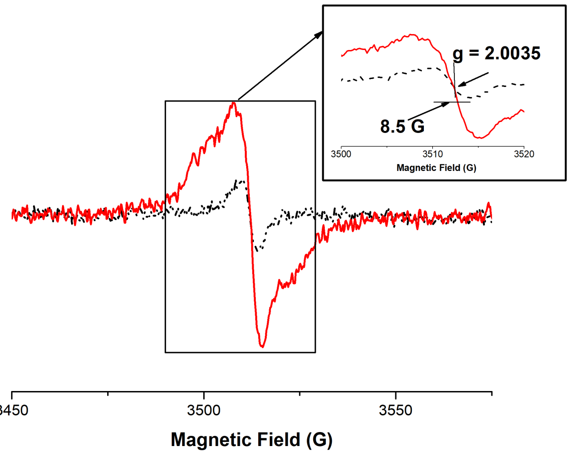


**Figure 2.** ESR spectra of rice: un-irradiated (dashed line) and irradiated (solid line). An inset magnify the central part..

## 2. Experimental Method

Riceberry rice grain was procured from Rice research Center, Kasetsart University, Thailand. It was kept away from light and humidity until the time of experiment. The irradiation process was performed at the Office of Atoms for Peace, Bangkok, Thailand. The 100-g pack of RB rice samples were irradiated in a GammaCell 220 Excel Irradiator with Co-60 at 1 Gy/s dose rate. Prior to the irradiation the routine dose mapping was carried out to ensure the pre-defined dose. The total dose is one kGy.

The irradiated RB rice grain was then transferred for an electron spin resonant measurement in the Bruker ELEXSYS E500 EPR Spectrometer. Normal or un-irradiated RB rice grain was also measured for reference. The ESR measurement was operated with MW X-band of 9.8 GHz at 0.5 mW power and 1 G p-p modulation under ambient condition. ESR spectra were then analyzed using EasySpin on MATLAB [8].

## 3. Results and Discussion

### *3.1. Typical ESR Spectrum of Riceberry Rice*

Figure 2 shows ESR spectrum from irradiated and normal riceberry rice. ESR signal of irradiated rice is much stronger than the normal rice. Also, the irradiated rice exhibits more complex shape indicating more increase , large and strong ESR is a singlet shape as shown in the figure 2. The signal has some noise to ratio signals because results of signal sensitivity of sample surface. The spectral parameters of the native rice have g = 2.0035 with 8.5 line width. The two parameters are assigned in the figure 2.

In the opposite way, the irradiated riceberry rice gives different features in ESR spectrum (figure 2). The shape of irradiated samples are more complicated signal shape than the native rice. The ESR signal strength is higher and the line width of peak-to-peak is wider than the native rice.

### *3.2. Free Radical Analysis*

According to ESR spectrum of the native rice, this shows there is free radicals only one group in native sample while some of the previous study have not found [2]. However, there is no determination of free-radical structure in the native rice but believing that interaction between glucose and environment factor is origin of radical formation.

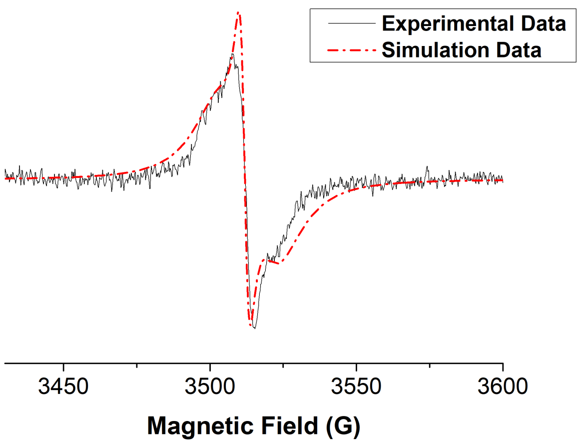


**Figure 3.** The simulated (— · · —) and and experimental ESR spectra (——) of the irradiated riceberry rice.

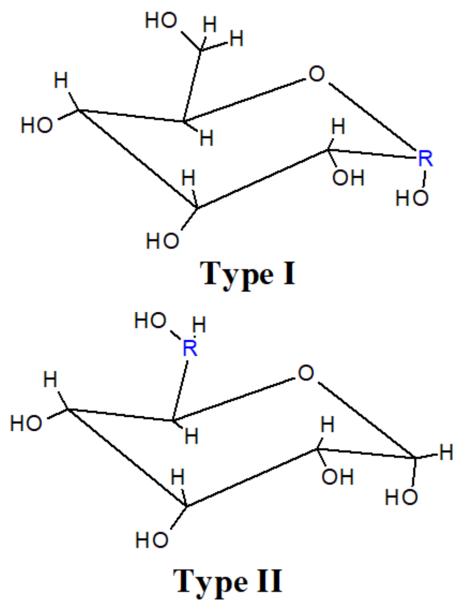


**Figure 4.** The possible free-radical structures that are induced by $\gamma$-ray irradiation. The structure in each type is corresponding to hydrogen abstraction on carbon atom position. The blue capital R refers to hydrogen abstraction of carbon atom.

In the same way, the irradiated rice is considered radical formation by using the model of glucose. The possible free-radicals from glucose are assigned from some study [2, 5]. Many methods for ESR spectrum analysis are used to interpretation free radical formation. The results form simulation by using various methods appear important ESR parameters, such as, Landé $g$ -factor and hyperfine splitting constant ($a$). These parameters are corresponding to three-free radical types on $\alpha$-D glucopyranose. The three free-radical types are from dependence on C-atom positions. These free radical structures from simulation give a detail that specified in each other as the Table 1.

Table 1 shows three propable free radicals; type I, II and III.The free radical type I is from hydrogen subtraction of C(1) on the pyranose ring. This radical exhibit doublet spectrum because the effect of nuclear spin of three protons nearby the C(1) (see figure 4). While the

**Table 1.** The ESR spectrum parameters from calculation for the three free-radicals structures in glucose. The estimated error of each parameters is absent because determination from the required software.

| Spectral Parameters | Value of the Spectral Parameters for Three-Free Radicals | | |
|---|---|---|---|
| | Type I | Type II | Type III |
| Line width (G) | 1.47 | 1.57 | 0.47 |
| Relative Amount | 88.50% | 2.21% | 9.29% |
| $g$-value | $g_x = 2.0020$<br>$g_y = 2.0032$<br>$g_z = 2.0042$ | $g_x = 2.0023$<br>$g_y = 2.0032$<br>$g_z = 2.0038$ | $g_x = 2.0023$<br>$g_y = 2.0023$<br>$g_z = 2.0023$ |
| Hyperfine Splitting Constants (G) $(a_x, a_y, a_z)$ | $a_{H,1}$ = (0.83, 1.30, 5.19)<br>$a_{H,2}$ = (0.52, -0.85, -1.44)<br>$a_{H,3}$ = (3.46, 4.77, 11.57)<br>$a_{H,4}$ = (0.52, -1.12, -1.79) | $a_{H,1}$ = (16.72, 18.29, 25.12)<br>$a_{H,2}$ = (-0.99, -6.58, 9.76)<br>$a_{H,3}$ = (-0.23, -0.91, 3.50)<br>$a_{H,4}$ = (-0.81, -0.88, 1.16) | |

radicals on C(6) is from proton subtraction on C(6) and four other equivalent protons nearby C(6) are affected. Therefore, the spectral of type II should be either doublet or triplet. The free radical type I and II may occurred while irradiating. Anyway, the singlet spectrum providess additional structure of free radical Type III.

## 4. Conclusion

Using ESR spectroscopy, we have measured on the irradiated riceberry rice and the native rice. The irradiated rice gives complicated shapes with three possible free-radicals. The free radicals type I and II give the same results on C(1) and C(6). While the free radicals on C(6) refers to hydroxyl group. The origin of all free radicals is hydrogen subtraction but the effect of hyperfine coupling of protons and C-atom gives difference ESR patterns.


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