

# Ultra-weak bioluminescence and vigour of irradiated rice

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**Abstract:** Detection of the irradiation dose and the vigour of irradiated rice based on the ultra-weak luminescent analysis is one of the promising analytical detection methods. Rough rice and head rice flour were used in this research for ultra-weak luminescent analysis. The bioluminescence intensity of the rough rice was different at varying irradiation doses and storage time. The trend of the differences was consistent with the germination rates of the irradiated rough rice. The changes of the bioluminescence intensity and the germination rate of the irradiated rough rice at diverse irradiation doses and storage time were due to the damage to the rice embryo caused by irradiation and the self-repair function of the embryo during storage. As a result, the ultra-weak luminescent analysis cannot detect the dose of the irradiation treatment on rice, but it can be used to detect the vigour of the irradiated rice. Experimental results show that the irradiation dose has a highly significant effect on the bioluminescence intensity of the rough rice flour when sucrose was added.

**Keywords:** ultra-weak luminescence, vigour, gamma irradiation, rice

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## 1 Introduction

Gamma irradiation has been used in the sterilization and pest control of agricultural products to prolong shelf-life and reduce health hazards<sup>[1]</sup>. Foods treated with irradiation of 10 kGy or less are confirmed to be wholesome, safe and nutritionally adequate for human consumption<sup>[2,3]</sup>. However, labelling is required for the consumers' freedom of choice<sup>[4]</sup>. At the international conference "The Acceptance, Control of and Trade in Irradiated Food", it was recommended that the compliance with labelling regulations should be checked directly for food products<sup>[5]</sup>, which means that the analytical detection method of irradiation treatment has to

be improved and new methods need to be developed<sup>[1]</sup>.

Luminescence analysis is one of the analytical detection categories that includes thermoluminescence analysis, ultra-weak bioluminescent analysis, chemiluminescence analysis, photoluminescence analysis, and some others<sup>[6]</sup>. Ultra-weak bioluminescence is a naturally occurred phenomenon of life. It is defined as an enzymatic chemiluminescent reaction with high quantum yield<sup>[7]</sup>. Previous studies<sup>[8-10]</sup> showed that gamma irradiation might change the structure of large molecules (e.g. starch, protein and fat), and consequently influence the drying characteristics and nutritional quality of rice. The change in molecular structure can result in enzymatic chemiluminescent reaction<sup>[11]</sup>. It is fair to hypothesize that the ultra-weak bioluminescence intensity of head rice (rice kernel without embryo) is correlated to the irradiation dose. The detection of the irradiation treatment in agricultural products based on ultra-weak bioluminescence may be feasible. However, few studies were reported on detection of irradiated rice by the ultra-weak bioluminescence.

The ultra-weak bioluminescent analysis is also an

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effective method for determining the degree of freshness of chicken eggs<sup>[12]</sup>, the singlet oxygen generation in germinating soybean<sup>[13]</sup>, and the number of bacteria<sup>[7]</sup> and fungi<sup>[14]</sup>. The vigour of life of rough rice with embryo may also be detected with ultra-weak bioluminescent analysis. It has been proved that the gamma irradiation could affect the vigour of agricultural products<sup>[15,16]</sup>. However, the few studies on ultra-weak bioluminescent analysis on detecting the vigour of irradiated rice were found.

The objectives of this study were to: (1) find the relationship between the ultra-weak bioluminescence intensity and the vigour of irradiated rough rice; (2) study the relationship between the ultra-weak bioluminescence intensity of head rice flour and the irradiation dose.

## 2 Materials and methods

### 2.1 Rough rice

A late-cropping season rough rice named Zhenong 1, was harvested in October, 2005 from the experimental farm of Agronomy, Zhejiang University, and used for this study. After the irritation treatment, all the rough rice samples were dried in an oven at 50°C to the safe moisture content of 13.5%±0.1% (dry basis) and then stored for 0 (non-storage), 1, 1.5, and 2 years under a commercial storage condition at (10±1)°C and 50%–55% relative humidity.

### 2.2 Gamma irradiation

Rice samples were exposed to 60°C source at ambient temperature at the Institute of Nuclear-agriculture Sciences, Zhejiang University. The irradiated doses for rough rice samples were 0 kGy (non-irradiation), 2 kGy, 5 kGy, 8 kGy, and 10 kGy respectively, with dose rate at 1 kGy/h. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) and the dosimeters were calibrated as per the International standard set by the International Atomic Energy Agency (Vienna, Austria)<sup>[11]</sup>.

### 2.3 Head rice flour

After gamma irradiation and drying, half of the rough rice samples were dehulled in a Satake dehuller and milled in a Satake TM-05 Grain-testing mill (Satake Co.,

Japan). The resulting milled rice was then ground in an Udy cyclone mill (Satake Co., Japan) with a 100-mesh sieve for study of the relationship between ultra-weak bioluminescence intensity of head rice flour and the irradiation dose. The head rice flour samples were also stored for 0 (non-storage), 1, 2, and 3 years under the same conditions as rough rice samples.

### 2.4 Ultra-weak bioluminescent detection

Preliminary experiments showed that 10 g in sample weight (including grain and flour) was enough to detect the ultra-weak bioluminescence<sup>[15,16]</sup>. In this study, the ultra-weak bioluminescence of grain and flour samples were measured with a BPCL ultra-weak luminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, China) at the room temperature of (30±1)°C. In each experiment, each sample was put in a special experimental vessel (the appurtenance of BPCL ultra-weak luminescence analyzer) for measuring the ultra-weak luminescence. The values of the ultra-weak bioluminescence of samples were calculated by subtracting the ultra-weak luminescence of the vessel from the total ultra-weak luminescence of the sample and the vessel<sup>[17]</sup>. The experiment was conducted with five replicates.

### 2.5 Vigour of rice and Germination test

One-hundred-grain seeds were symmetrically placed on two pieces of filter paper, which had steeped in water for one hour, in a 15-cm-diameter Petri dish. Seeds were incubated for 12 h in light and another 12 h in darkness in this sequence for 14 days in growth chambers at 30°C constantly (GB/T 3543.4-1995, National Standard of China). Seed germination rate was determined at the 14th day. The germination tests were carried out after storage for 0 (non-stored), 1, 1.5 and 2 years. Seed samples were considered as germinated seeds when the root was 5 mm long.

Experiment for each sample had three replications. The standard deviation and ANOVA analysis was calculated using SAS software (SAS Institute Inc. 1999).

## 3 Results and discussion

### 3.1 Effect of dose on the ultra-weak bioluminescence intensity for rough rice

The effect of dose on the ultra-weak bioluminescence

intensity for rough rice after different storage time was shown in Figure 1. It was obvious that the bioluminescence intensity of the rough rice was different at varying irradiation doses and storage time.

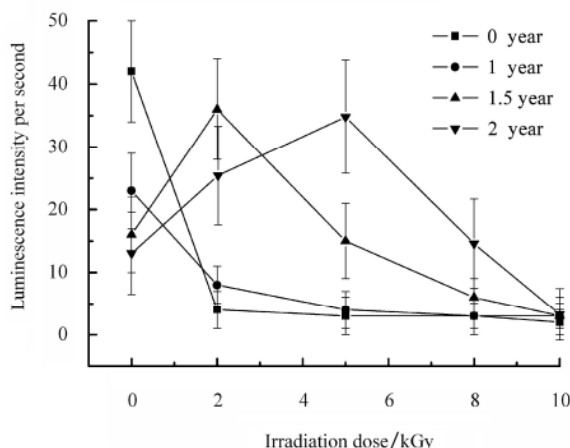


Figure 1 Effect of dose on the ultra-weak bioluminescence intensity of the irradiated rough rice after different storage time

With the increasing storage time, the bioluminescence intensity of the non-irradiated rough rice decreased, but the bioluminescence intensities of the rough rice irradiated with 5 kGy and 8 kGy increased. The bioluminescence intensity of the 2 kGy irradiated rough rice had a higher value after 1.5 year storage. The results may be because that the 2 kGy irradiated rough rice had a higher vigour after 1.5 year storage. The bioluminescence intensity of 10 kGy irradiated rough rice did not show a significant difference over four years of storages.

### 3.2 Relationship between the ultra-weak bioluminescence intensity and the vigour of irradiated rough rice

It was known that the embryo in the rough rice had different levels of life activity for germination. The life activity of rough rice was proved to be different with the diverse irradiation doses<sup>[15]</sup>. The changing of ultra-weak bioluminescence intensity of the rough rice (Figure 1) may be affected by the different life activity of the rough rice.

The effect of irradiation dose on the germination rate of the irradiated rough rice after different storage time was shown in Figure 2. The trend of germination rate for the irradiated rough rice at different doses and after different storage time was similar to that of bioluminescence intensity for rough rice samples at

different doses after different storage time.

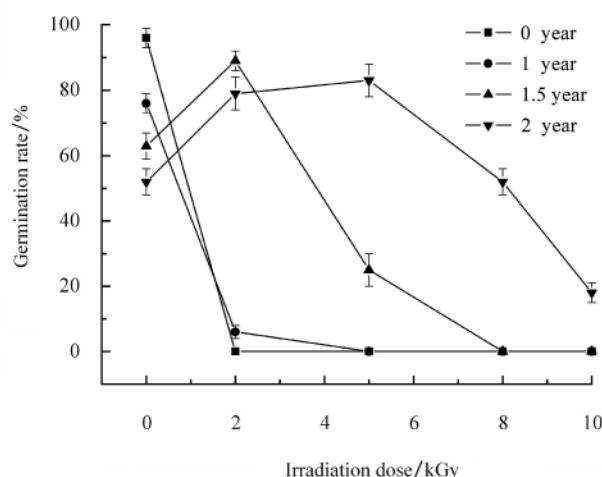


Figure 2 Effect of dose on the germination rate of the irradiated rough rice after different storage time

With the increasing storage time, the germination rate and the bioluminescence intensity for the non-irradiated rough rice decreased (Figure 1). The bioluminescence intensity had a significant positive correlation with the life activity of living organisms<sup>[7,13]</sup>. For example, the germination rates of the 5 kGy and the 8 kGy irradiated rough rice samples increased with the increasing storage time because of the increasing life activity. The change of life activity for the irradiated rough rice at different storage time may be due to the interaction between the self-repair function which increases the life activity and the respiration which decreases the life activity of the irradiated organism<sup>[18,19]</sup>. The life activity of the rough rice decreased evidently after irradiation and increased to a higher level after 1.5 years and 2 years of storage respectively due to the self-repair during storage. With the change of the life activity and the germination rate of the irradiated rough rice, the bioluminescence intensity of irradiated rough rice samples changed accordingly (Figure 1). The germination rates of the 10 kGy irradiated rough rice sample were all very low during storage, which is the reason for the insignificant difference in bioluminescence intensity of the 10 kGy irradiated rough rice among different storage time.

After 0 and 1 year of storage, the high germination rates (>75%) appeared for the non-irradiated rough rice because of its higher life activity; but the germination rate was very low when the dose was higher than 2 kGy due

to the low life activity.

This is because of the effect of irradiation on rice embryo that the embryo is damaged by  $\gamma$ -ray<sup>[7,15]</sup>. After one year storage, the self-repair function of the irradiated rough rice did not show. After 1.5 and 2 years of storage, the higher germination rates of the rough rice appeared at 2 kGy and 5 kGy, respectively. It was because that the self-repair of rough rice sample occurred with the increasing storage time and had a higher degree at 2 kGy and 5 kGy after 1.5 and 2 years of storage respectively. These results demonstrated the correlations of the change of bioluminescence intensity of the irradiated rough rice, the irradiation dose, and the storage time (Figure 1).

### 3.3 Effect of dose on the ultra-weak bioluminescence intensity of the head rice flour

The embryo was taken out and the head rice flour was used to detect the effect of dose on the ultra-weak bioluminescence intensity. The effect of dose on ultra-weak bioluminescence intensity of head rice flour after different storage time was shown in Figure 3. The bioluminescence intensity of the head rice flour increased with the increasing dose. The results could be predicted due to the changing of molecular structure caused by irradiation<sup>[7,13]</sup>.

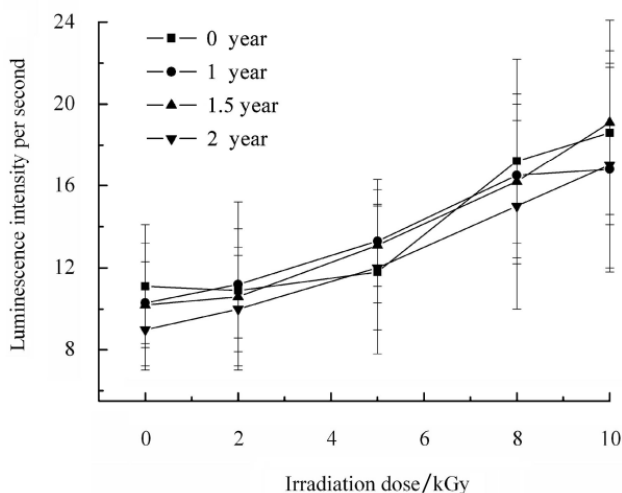


Figure 3 Effect of dose on the ultra-weak bioluminescence intensity of the irradiated head rice flour after different storage time

The bioluminescence intensity of the head rice flour was not significant affected by the storage time or the irradiation dose ( $p > 0.1$ , Table 1).

**Table 1** Effect of inducement on bioluminescence by ANOVA analysis

	No addition in head rice flour	Water was added	Sucrose was added
Effect of dose	$P > 0.1$	$p < 0.05$	$p < 0.01$
Effect of storage time	$p > 0.1$	$p > 0.1$	$p > 0.1$

Note: Irradiation dose: 0 kGy, 0.6 kGy, 1.5 kGy, 2.4 kGy and 3 kGy. Storage time: 0 year, 1 year, 1.5 year and 2 year.

### 3.4 Inducement of bioluminescent

The ultra-weak bioluminescence intensity of the head rice flour was proved to be a possible way to predict the dose of irradiation on the rough rice. But the lower correlation between the bioluminescence intensity of the head rice flour and the dose of irradiation indicated that predicting the dose was not feasible yet.

Water as an evocator was added in the head rice flour to make the mixture 15% in moisture content. The mixture was added to the injection aperture of the BPCL ultra-weak luminescence analyzer during the ultra-weak bioluminescent detection. When water was used as a revulsant of the ultra-weak luminescence, the effect of the dose on the ultra-weak bioluminescence intensity of the head rice flour at different storage time was shown in Figure 4. Compared with Figure 3, a similar result could be found. The irradiation dose has a significant effect on the bioluminescence intensities of the head rice flour when water was added ( $p < 0.05$ , Table 1).

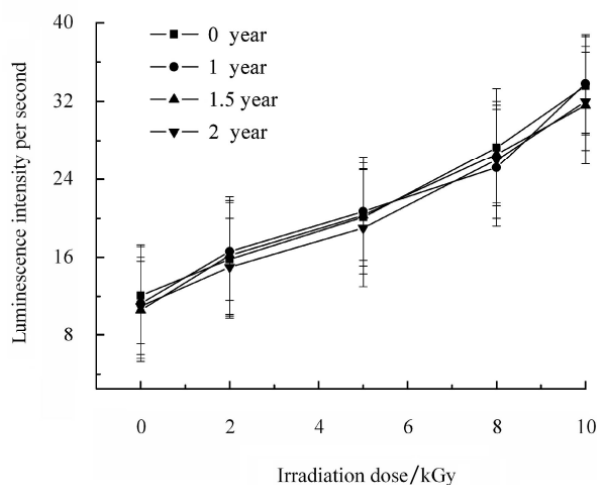


Figure 4 Effect of dose on the ultra-weak bioluminescence intensity of the irradiated head rice flour mixed with water after different storage time

Sucrose solution as an evocator was added to the head

rice flour from the injection aperture of the BPCL ultra-weak luminescence analyzer during ultra-weak bioluminescent detection. When sucrose solution was used as a revulsant of ultra-weak luminescence, the effect of dose on the ultra-weak bioluminescent intensity of the head rice flour at different storage time was shown in Figure 5. Comparing Figure 3, 4 and 5, a most distinct trend could be seen from Figure 5, and the irradiation dose has a highly significant effect on the bioluminescence intensity of head rice flour when sucrose was added ( $p < 0.01$ , Table 1).

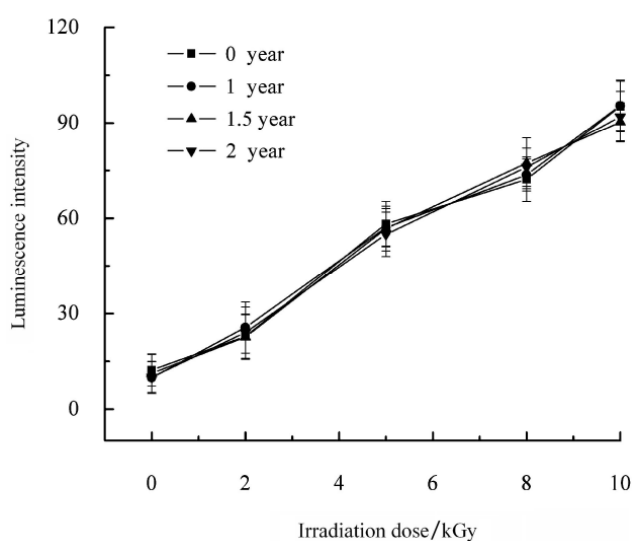


Figure 5 Effect of dose on the ultra-weak bioluminescence intensity of irradiated head rice flour mixed with sucrose solution after different storage time

## 4 Conclusions

The bioluminescence intensity and the vigour (germination rate) of rough rice samples were different with the increase of the storage time and the irradiation dose. Thus the ultra-weak luminescent analysis could be used to distinguish the vigour difference of rough rice seeds.

The bioluminescence intensity of head rice flour increased with the increasing dose of the irradiation treatment. The ratio of bioluminescence intensity versus irradiation dose was not significantly affected by different storage time. The irradiation doses have a highly significant effect on the bioluminescence intensity of the head rice flour when sucrose was added.

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