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## Effect of temperature and relative humidity on chemical analysis of red rice germination

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**Abstract.** Germinated rice (GR) is known as nutritional rice due to the higher content of nutrients compared to the conventional brown rice, such as antioxidant i.e. *gamma-amirobutyric acid* (GABA), gamma-oryzanol, phenolic, and essential amino acid (lysine and niacine), 3 times vitamin B<sub>1</sub> and B<sub>2</sub>, some minerals, and 4 times the dietary fiber. Generally, GR is made from germinated rice. However, in this research, the GR was made from germinated grain. The purpose of this study was to analyze the chemical properties (protein, carbohydrate, ash, fat, minerals (Fe, Mg, Mn), vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>), and antioxidant activity of GR which that germinated in various temperature and relative humidity (RH) condition. The method used in this research divided into two stages: first, determining the fastest germination time of temperature (room, 20 °C, and 30 °C) and RH (room, 70, 80, and 90%) treatments. The second, determining the effect of the best treatment on the chemical analysis germinating red rice. This study used a complete randomized design method with two replications. The results showed that temperature and RH influenced the chemical analysis of red rice germinated by increasing vitamin B<sub>6</sub>, ash content, carbohydrate content (room and 20 °C, 90% RH treatments), and fat content (30 °C, 90% RH treatment). But decreasing the protein, vitamin B<sub>1</sub>, and some minerals such as Fe, Mg and Mn compared to non-germinated red rice. In addition, all treatments had strong antioxidant activity.

### 1. Introduction

Red rice is one of the varieties of rice that colour red by its anthocyanin content. Red rice contains more nutrients than white rice (milled and polished rice). Every 100 g of red rice contains protein 7 g, iron 5.5 mg, zinc 3.3 mg, and fiber 2 g [1]. High quality and nutrition rice is produced by several combinations of processing, one of them is germinated rice. Germinated rice (GR) is a functional food that contains *gamma-amirobutyric acid* (GABA), gamma-oryzanol, phenolic, and essential amino acid (lysine and niacine), 3 times vitamin B<sub>1</sub> and B<sub>2</sub>, some minerals, and 4 times dietary fiber [2]. Generally, GR is made from germinated rice. However, in this research, the GR was made from germinated grain. In order to water soluble nutrients contained in the husk's skin can be absorbed into the rice during the soaking and germination process [3,4]. Germination is a sign of an early seed life. When seeds are germinated, many nutritional compositions are prepared for sprout growth [5]. One of the factors that influence the quality of seed germination is soaking water temperature and relative humidity (RH) [6]. Hence, the purpose of this study was to analyze the chemical properties (protein, carbohydrate, ash, fat, minerals (Fe, Mg, Mn), vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>), and antioxidant activity of GR which that germinated from various temperature and relative humidity (RH) condition.



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## 2. Materials and methods

### 2.1. Materials

The fresh harvested grain was purchased from a farmer at Sidrap, South Sulawesi, Indonesia. Chemical reagents for analysis such as DPPH, HCL, H<sub>2</sub>SO<sub>4</sub>, and CH<sub>3</sub>COONa were purchased from a chemical market in Makassar, Indonesia.

### 2.2. Methods

The method used in this research divided into two stages: first, determining the fastest germination time of temperature (room, 20 °C, and 30 °C) and RH (room, 70, 80, and 90%) treatments. The second, determining the effect of the best treatment on the chemical analysis germinating red rice.

*2.2.1. Sample preparation.* Dry grain (14% water content) soaked in the water (1:2 weight/volume) at temperature (room, 20 °C and 30 °C) and RH (room, 70, 80, and 90%) for 48 hours. After the water is drained, each sample is then germinated in sacks with the same temperature and RH until it sprouts with an average length of 1 cm. The germinated grain then be dried until water content reaches 14% for the milling process and analyzed for the chemical content.

*2.2.2. Determination of protein.* Protein content was determined by the Kjeldahl method [7]. The protein is expressed as the percentage of the total protein.

*2.2.3. Determination of fat.* Fat content was analyzed by using the Soxhlet method [7]. Fat is expressed as the percentage of fat.

*2.2.4. Determination of carbohydrate.* Measurement of the total carbohydrate content in a sample is calculated based on calculation (in %) [8]

$$\% \text{ Carbohydrate} = 100\% - \% (\text{protein} + \text{fat} + \text{ash} + \text{water})$$

*2.2.5. Determination of ash.* Five grams of sample were added into the metal cup, and the sample was heated at 75 °C for 3 hours. After 3 hours, the sample is then cooled in a desiccator for 10 minutes then weighed. Ash content is expressed as the percentage of ash [7].

*2.2.6 Determination of vitamin B<sub>1</sub>, B<sub>2</sub> and B<sub>6</sub>.* Vitamin B<sub>1</sub>, B<sub>2</sub>, and B<sub>6</sub> were analyzed by using the HPLC method using C18 column, UV-Vis detection at λ 254 nm for vitamin B<sub>1</sub>, vitamin B<sub>2</sub>, and vitamin B<sub>6</sub> [9].

*2.2.7. Determination of minerals (Fe, Mangan, Magnesium).* Minerals of Fe, Mn, and Mg analyzed by using Atomic Absorption Spectrophotometer (AAS) method at wavelength λ 285.2 nm for Fe, λ 279.5 nm for Mn, and λ 766.5 nm for Mg [7].

*2.2.8. Determination of antioxidant activity.* Analysis of antioxidant activity was measured by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Absorbance values were measured using UV-Vis spectrophotometer at a wavelength of 515 nm [10].

### 2.3. Statistical analysis

The experiment was designed by using factorial randomized complete random (RAL) with two replications, and the data were analyzed by using analysis of variance (ANOVA), if the data significantly different, then proceed with the Duncan test using SPSS ver. 22.

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### 3. Results and discussion

#### 3.1. Time of red rice germination

Table 1 showed the time of red rice germination to reach a germination length 1 cm. The results showed the lower temperature and RH, the longer the time for the grain to germinate, and reverse the higher the temperature and RH, the faster the grain will germinate. The higher temperature of grain germination, the faster the grain metabolic activity such as respiration and germination [11]. The best treatment for each group was 20 °C, RH 90%; 30 °C, RH 90%; and room temperature and RH with the time of germination 70; 48 and 48 hours, respectively.

**Table 1.** Time of germination of all treatments to reach a germination length 1 cm.

Germination Treatments T (°C), RH (%)	t (hours)
20 , 70	76
20 , 80	76
20 , 90	70
30 , 70	60
30 , 80	56
30 , 90	48
Room	48

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#### 3.2. Protein content

The protein content of germinated red rice was analyzed. Figure 1 showed protein content for all treatments decreased compared to non-germinated rice. However, based on the Duncan test were found that the amount of protein of non-germinated red rice was not different from protein content of room and 30 °C, 90% RH treatments, but the protein content of 20°C, 90% RH treatment was different from protein content of non-germinated red rice. Decreasing protein level due to the influence of a long germination process that is 70 hours, so that the process of respiration and proteolysis by microorganisms through a complex enzyme system breaks down protein into simpler compounds [12].

The result of this study was the same as from other research [12]. Protein from some varieties of germinated rice tends to decrease. But differ from germinated white rice in which showed increased protein content at 1 cm germination length [4] and a small increased at germinated red rice from 6.06% to 6.12% [12]. The results assumed that the increase was due to the synthesis of enzyme protein during imbibition in the germination process [13,14]. Based on statistical analysis, temperature treatment significantly affected the protein content at the level 5%.

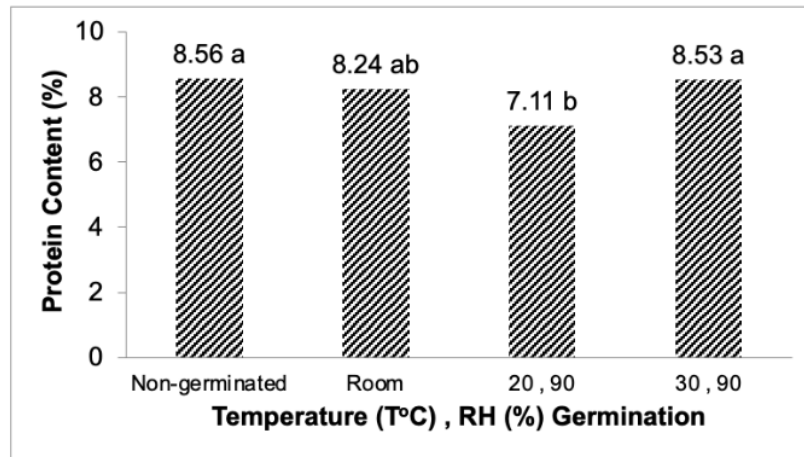


Figure 1. Protein content of germinated red rice in different treatments.

10. Fat content

As shown in figure 2, the fat content of treatments at room and 20 °C, RH 90% tends to decrease. But the fat content of the treatment 30 °C, RH 90% tend to increase. However, based on the Duncan test, the fat content was not different from non-germinated rice and at room temperature and RH treatments.

The results of this study were the same as from germinated red rice in that fat tend to decrease with an increase in the time of germination [12], because fat was used as the major source of carbon for seed growth [13], and Hahm *et al.* (2008) [15] also suggested that fatty acids are oxidized to carbon dioxide and water to generate energy for germination. Based on statistical analysis, temperature treatment and relative humidity significantly affected the fat content at the levels 1% and 5%.

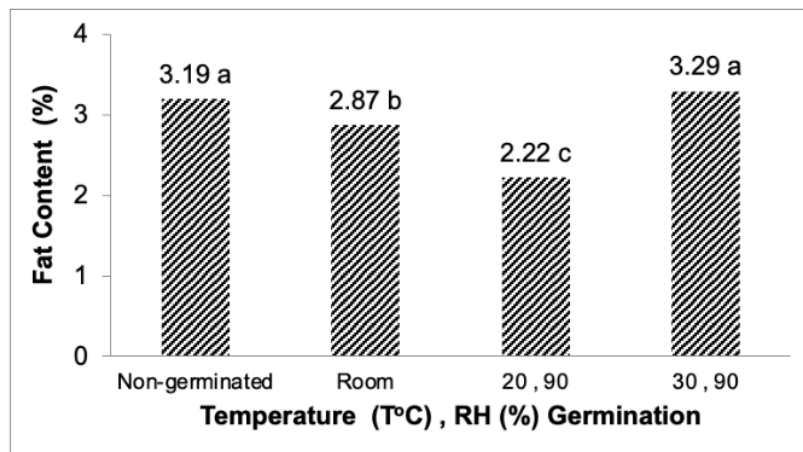


Figure 2. Fat content of germinated red rice in different treatments.

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3.4. Carbohydrate content

As shown in figure 3, carbohydrate content for room and 20 °C, RH 90% treatments tend to increase. But the treatment 30 °C, RH 90% tend to decrease, however, based on the Duncan test the carbohydrate content was not different from non-germinated rice. Increased carbohydrate levels due to its was used as source of energy for embryonic growth which could explained the changes of carbohydrate content after germination. Additionally,  $\beta$ -amylase activity that hydrolyzes the starch into simple carbohydrates was increased [16]. Based on statistical analysis, temperature treatment significantly affected the carbohydrate content at the level 5%.

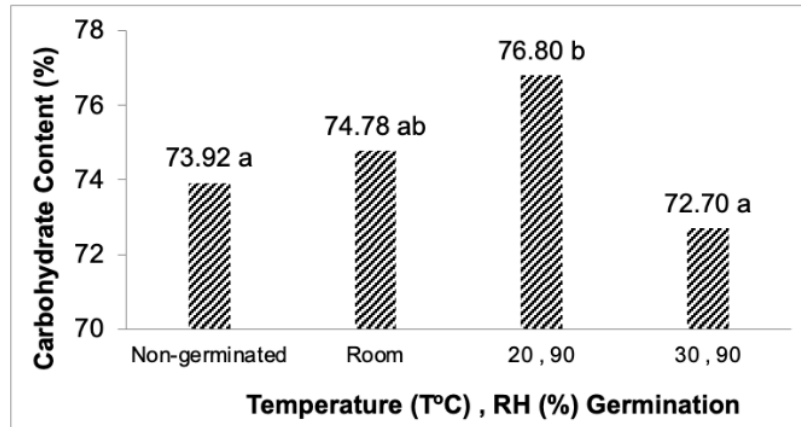


Figure 3. Carbohydrate content of germinated red rice in different treatments.

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3.5. Ash content

As shown in figure 4, Ash content for all treatments increased compared to non-germinated rice. Increased ash levels due to the effect of soaking and germination. During soaking, minerals in the hush are absorbed into the rice [4], and during germination, some enzymes will be active, for example, an enzyme used for growing sprouts. The enzyme will free the bonds between minerals and proteins so that the availability of minerals during germination will increase [17]. Based on statistical analysis, temperature treatment significantly affected the ash content at the level 5%.

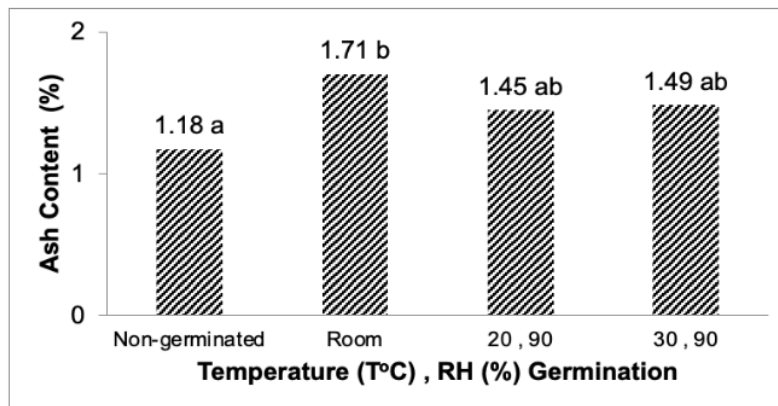


Figure 4. Ash content of germinated red rice in different treatments.

3.6. Vitamin B<sub>1</sub> content

Vitamin B<sub>1</sub> (thiamine) is a group of water-soluble vitamins, as shown in figure 5, vitamin B<sub>1</sub> content for all treatment decreased than non-germinated rice. Decreased vitamin B<sub>1</sub> levels due to the influence by soaking, during soaking vitamin B<sub>1</sub> soluble in water. Decreased of vitamin B<sub>1</sub> was also assumed that vitamin B<sub>1</sub> is a precursor of the thiamine diphosphate cofactor required by various enzymes that participate in the metabolism of carbohydrates and amino acids [18]. The result of this study is different from germinated white rice in that thiamine tends to increase [4]. Based on statistical analysis, temperature treatment and relative humidity significantly affected vitamin B<sub>1</sub> at a level 5 %.

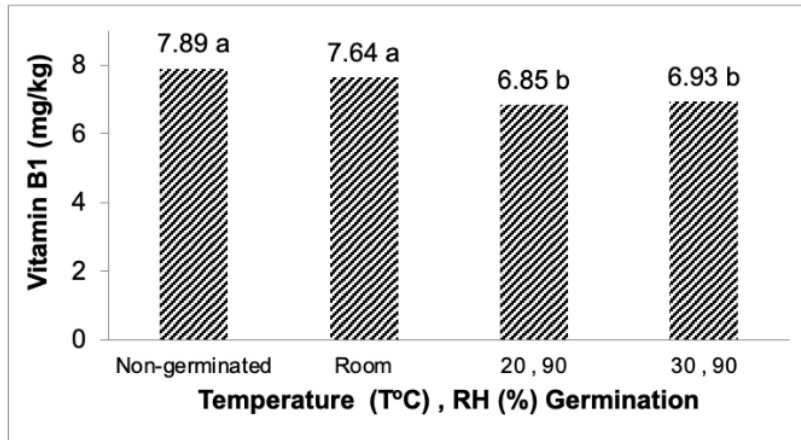


Figure 5. Vitamin B<sub>1</sub> content of germinated red rice in different treatments.

3.7. Vitamin B<sub>6</sub> content

As shown in figure 6, Vitamin B<sub>6</sub> (Pyridoxine) content for all treatment increased to almost 3 times than non-germinated rice, it is because effect of germination. During germination, vitamin B<sub>6</sub> is needed for germination process [19]. Based on statistical analysis, temperature treatment and relative humidity significantly affected the vitamin B<sub>6</sub> content at the level 1%.

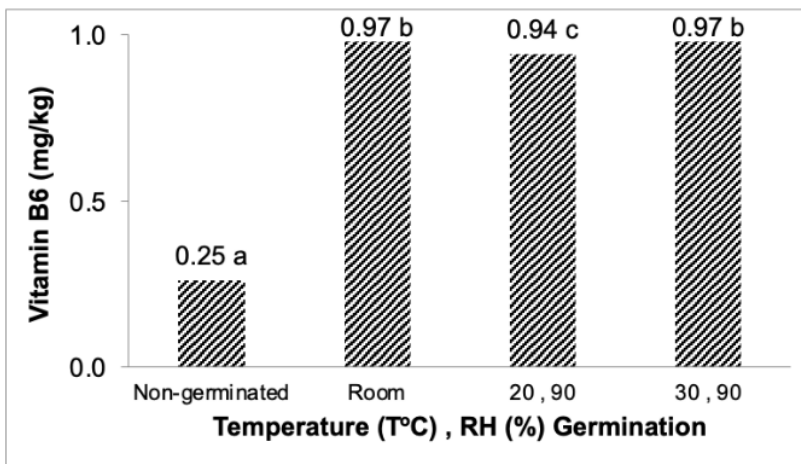


Figure 6. Vitamin B<sub>6</sub> content of germinated red rice in different treatments.

3.8. Vitamin B<sub>2</sub> content

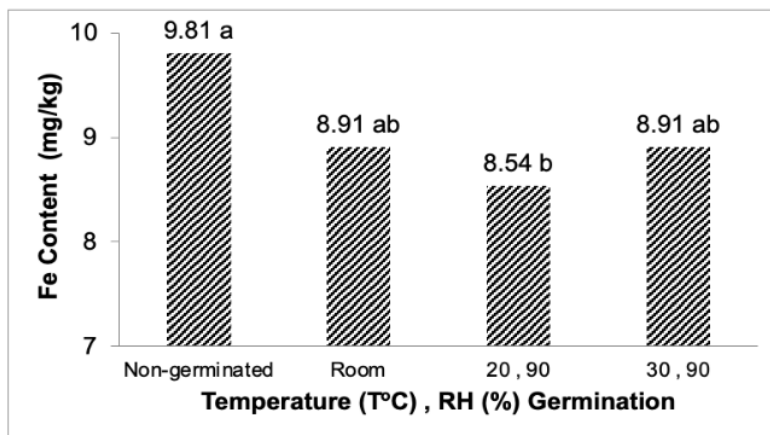
As shown in table 2, vitamin B<sub>2</sub> for all treatments less than 0.25 mg/kg or non-detected, non-detected because a variety of red rice used in this research content little of vitamin B<sub>2</sub>.

**Table 2.** Vitamin B<sub>2</sub> content of germinated red rice.

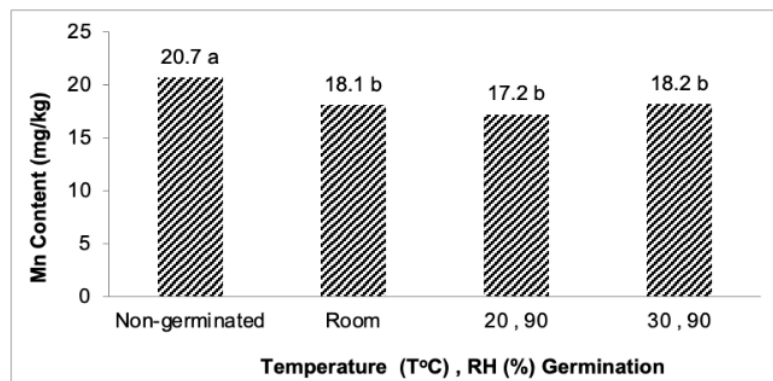
Treatments	Vitamin B <sub>2</sub> (mg/kg)
Non-germinated	< 0.25
Room	< 0.25
20, 90	< 0.25
30, 90	< 0.25

3.9. Mineral Fe, Mg and Mn content

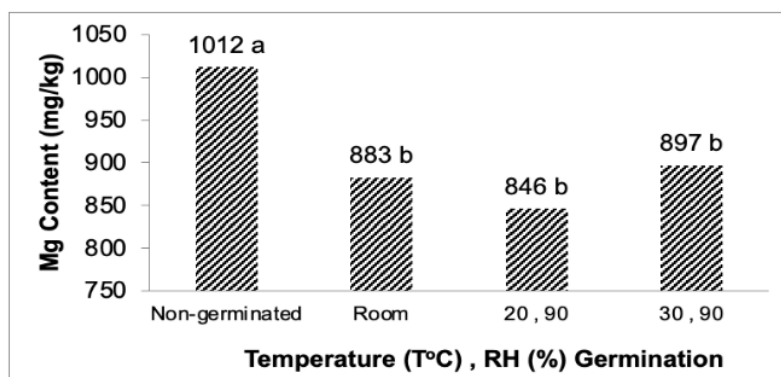
As shown in figure 7, Minerals Fe, Mg, and Mn for all treatments decreased compared to non-germinated rice. Decreased all mineral levels due to the dissolution of Fe, Mg, and Mn into the water during soaking. The result of this study is the same from germinated white rice in that Fe and Mg tend to decrease [4]. Based on statistical analysis, temperature treatment and relative humidity significantly affected the Fe content at the level 1% and 5%; and significantly affected the Mg and Mn content at the level 5% for temperature and humidity treatment.



(a)



(b)



(c)

**Figure 7.** Minerals (Fe, Mg, Mn) content of germinated red rice in different treatments.

**3.10. Antioxidant activity**

As shown in figure 8,  $IC_{50}$  is a parameter showing antioxidant activity. Antioxidant activity germinated red rice tends to decrease, however, the activity is still at a strong antioxidant activity level (50-100 ppm). The compound is said to have very strong antioxidant activity if the  $IC_{50}$  value is less than 50 ppm, strong if the  $IC_{50}$  value is between 50-100 ppm, moderate if the  $IC_{50}$  value is between 101-250 ppm, it is weak if the  $IC_{50}$  value is between 250-500 ppm and is not active if the  $IC_{50}$  value is more from 500 ppm [20]. Based on statistical analysis, temperature treatment and relative humidity significantly affected the antioxidant activity at a level 1 %.

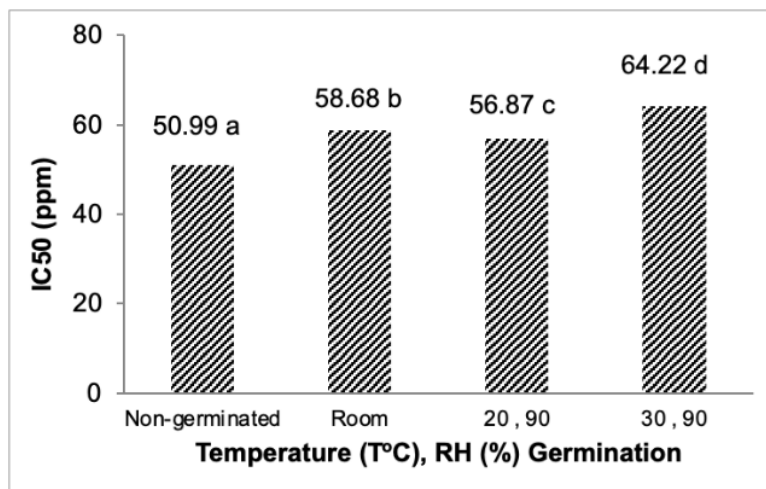


Figure 8. IC<sub>50</sub> (ppm) value of germinated red rice in different treatments.

#### 4. Conclusions

Temperature and humidity affect the germination process. The lower the temperature and humidity, the longer the germination time and reverse. Grain germination from different temperature and relative humidity increase vitamin B6, ash content, carbohydrate content for room and 20 °C,90% RH treatments, and increase fat content for treatment 30 °C,90% RH. But decrease some nutrients such as protein, vitamin B1 and some minerals such as Fe, Mg and Mn compared to non-germinated red rice. All treatments had strong antioxidant activity because they have IC<sub>50</sub> between 50-100 ppm.

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