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## Analysis of biochemical and bioactive content of red rice cv Sidikalang through gamma ray irradiation induction (M4)

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### ABSTRACT

Red rice derives its characteristic color from anthocyanin pigments located in the pericarp and outer endosperm layers, which contribute to its biochemical and bioactive properties. Gamma-ray irradiation is widely applied as a mutation induction technique to enhance genetic variability and improve functional traits in crop plants. This study aimed to evaluate the biochemical and bioactive content of the red rice cv Sidikalang following gamma-ray irradiation. The experiment was conducted at the Central Laboratory, Faculty of Agriculture, Universitas Sumatera Utara, from October 2024 to February 2025, using a single-factor Randomized Block Design with irradiation doses of 0, 200, 400, and 600 Gy and three replications. Biochemical and bioactive parameters analyzed included carbohydrate, protein, fat, vitamin A, fiber, ash, water content, and anthocyanin. The results showed that gamma-ray irradiation had no significant effect on protein, fat, vitamin A, and carbohydrate content, but significantly affected water content, fiber, ash, and anthocyanin. Irradiation doses of 200-600 Gy resulted in significantly higher fiber and anthocyanin content compared to the control. These findings indicate that gamma-ray irradiation has the potential to enhance specific functional and nutritional traits of red rice, providing valuable insights for mutation breeding programs aimed at developing nutritionally improved rice varieties.

**Keywords:** red rice, bioactive, biochemistry, cultivar Sidikalang, gamma rays.



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### 1. Introduction

Rice (*Oryza sativa* L) is a staple food source in Indonesia, with an average household consumption of 81.52 kg/capita/year in 2022 [1]. The increasing global population is projected to escalate food demand, thereby necessitating more intensive rice cultivation systems with enhanced nutritional quality. Variations in rice characteristics, particularly grain pigmentation, have attracted increasing attention due to their nutritional potential.

Based on color (pigment), there are two (2) types of rice, namely pigmented (red and black rice) and non-pigmented (white rice). In general, Indonesian people cultivate and consume more white rice than red rice [2]. In white rice (non-pigmented), there is no accumulation of colored compounds, while pigmented rice has an accumulation of pigment compounds resulting in colored rice grains.

The bran layer on the seeds contains protein, fiber, oil, minerals, vitamins, and anthocyanin and proanthocyanidin compounds, which give rice bran its color [3]. Excessive milling and polishing processes can cause erosion of the rice caryopsis layer, resulting in a significant reduction in the content of bioactive compounds and their antioxidant capacity [4].

Pigmented rice contains phytochemical compounds that have antioxidant effects. Based on several studies, pigmented rice contains significantly higher levels of phenolic compounds compared to non-pigmented rice [5]. The red color found in red rice is due to the anthocyanin content found in the pericarp layer of the outer layer of the rice endosperm [6]. Anthocyanin is a phenolic compound included in the flavonoid group which plays an important role for the plant itself and is beneficial for human health [7]. The anthocyanin content in brown rice can function as an antioxidant, anti-mutagenic, hepatoprotective, anti-hypertension and anti-hyperglycemic. Brown rice also contains Gamma Amino Butyric Acid (GABA) fiber, carbohydrates, proteins, and essential fatty acids. The dietary fiber content of brown rice may be up to five times greater than that of white rice. This elevated fiber level contributes to reducing cholesterol concentrations by limiting the absorption of excess carbohydrates, fats, and proteins in the body [8].

Rice cultivars/varieties that produce red rice have different biochemical and bioactive contents from one another. Nutritional content of local red rice from North Kalimantan in several areas namely Klubir, Tanjung Palas, Pimping, Teras Baru and Selimau contains: carbohydrates 73.16-79.58%, fat 1.00-2.07%; protein 7.72-9.10%; crude fiber 0.24-1.77%; total ash 0.4-1.01% and antioxidant activity (IC50) 85.6-290.54 ppm [9]. These data are presented to illustrate the broad variability of nutritional and bioactive profiles in Indonesian local red rice germplasm, as comprehensive biochemical data on local red rice varieties from Sumatra remain limited. This highlights the importance of further studies on Sumatra-origin red rice cultivars, such as Sidikalang, to expand region-specific information and support the development of nutritionally improved local rice varieties.

Rice can be categorized according to its amylose content into the following groups: glutinous rice (0-5%), very low (5- 12%), low (12-20%), medium (21-25%), and high (25-33%) [10]. According to [11], good-tasting rice has a protein content of less than 7% and a fat content of around 3%. Fiber is an important component in the digestion process. The crude fiber component consists of cellulose, hemicellulose, and lignin. The ideal crude fiber is 36% [12].

Efforts to increase rice production and quality are certainly inseparable from the use of seeds. This is a target for rice plant breeders in several countries due to changes in lifestyle patterns. Upland rice cultivar Sidikalang is one of the local rice cultivars of North Sumatra. The cv Sidikalang produces red rice so it has great potential to be developed however, so far the author does not know how much bioactive content (anthocyanin) the cv Sidikalang contains, while the biochemical content is still limited to amino acid testing [13] One method that can be used to improve the diversity of plant characters (morphology, biochemistry and bioactive) is by mutation induction that can be done by using mutagens. There are two types of mutagens, namely: (1) physical mutagens, namely irradiation without using ionization (UV rays) and irradiation with ionization; (2) chemical mutagens, namely mutation induction using chemicals. Physical mutagens cause mutations at the chromosome level, while chemical mutagens cause mutations at the gene or nitrogen base level [14]. The success of a mutation depends on the tissue irradiated and the length of time of irradiation [15]. Induction of gamma ray irradiation (mutation) results in a heritable change that occurs in DNA. Testing of bioactive compound content and biological activity in several Thai rice varieties induced by gamma ray irradiation has been reported by [16]. This study aims to obtain the amount of biochemical and bioactive content of red rice cv Sidikalang induced by gamma ray irradiation in the M4 generation.

## **2. Materials and Methods**

### *2.1. Place and Time*

This research was conducted in the Central Laboratory of the Faculty of Agriculture, Universitas Sumatera Utara, Medan, from October 2024 to February 2025.

### *2.2. Materials and Tools*

Cultivar rice Sidikalang resulting from gamma ray irradiation (M4) with doses of 0 Gy (R0), 200 Gy (R1), 400 Gy (R2) and 600 Gy (R3) obtained from the results of the author's previous research, 95% ethanol, 1 N NaOH, ddH<sub>2</sub>O, Aquadest, 2% Iodine. K<sub>2</sub>SO<sub>4</sub>, HgO, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub>, NaOH-Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, HCl, H<sub>2</sub>SO<sub>4</sub> (0.255 N), NaOH (0.313 N), acetone, hexane, 0.05% BHT, 95% alcohol KCl buffer. Spectrophotometer, test tube, sieve, filter paper, blender, analytical balance, measuring cup, centrifuge, vortex, 100 ml volumetric flask, 1 ml pipette, 10 ml pipette, Kjeldahl flask, distillation apparatus, Erlenmeyer, UV-vis 1240, micro cuvette, 5 ml volume pipette, propipette, analytical balance meter, white tube, reflux, reverse condenser, crucible cup, desiccator, oven, basin, 500 ml glass beaker, 25 ml measuring cup, rubber plastic, label paper, analytical

balance, medium basin, filter cloth, stove, ruler, knife, panic, newspaper/cover paper, Bunsen, LAF, stirrer, evaporating cup, pipette.

### 2.3. Methods

This research used a one-factor Randomized Block Design (RBD), consisting of 4 levels of irradiation dose (R): 0 Gy (R0), 200 Gy (R1), 400 Gy (R2), and 600 Gy (R3), which were repeated three times. Biochemical content analysis includes: Carbohydrate analysis using the Kjeldahl method; protein analysis using the Kjeldahl method; analysis of fiber content using the Gravimetric method, AOAC; ash analysis; analysis of anthocyanin content using the pH differential method. This research consists of two stages:

#### 2.3.1. Preparation of rice flour samples

In mutation breeding studies, advanced generations are commonly used to reduce segregation effects and allow more reliable assessment of induced traits. Although some variability may still occur, the M4 generation is generally considered sufficiently stable for evaluating biochemical and bioactive characteristics. Red rice cv Sidikalang (M4) with irradiation dose treatment (0 Gy (R0), 200 Gy (R1), 400 Gy (R2), and 600 Gy (R3), the rice was milled to produce rice flour, then the rice from each treatment was blended to obtain rice flour, and then sieved through an 80 mesh.

#### 2.3.2. Analysis of the biochemical and bioactive content of Sidikalang rice consists of;

##### 2.3.2.1. Protein content analysis (Kjeldahl method) [17]

Protein content was determined using the Kjeldahl method, based on the conversion of nitrogen into ammonia. The procedure included the following stages:

##### a. Digestion

Approximately 2.0 g of sample was weighed into a Kjeldahl flask with boiling stones, 5 g K<sub>2</sub>SO<sub>4</sub>, HgO, and 30 mL concentrated H<sub>2</sub>SO<sub>4</sub>. The mixture was heated in a fume hood, initially with a low flame and then increased until the solution became clear green. The digested solution was then cooled with distilled water.

##### b. Distillation

The digested sample was transferred to a distillation apparatus. The flask and condenser were rinsed 5–6 times with distilled water to ensure complete transfer. NaOH solution was added, and ammonia was distilled into an Erlenmeyer containing boric acid and an indicator solution. The condenser was rinsed with distilled water, and the rinsate was collected in the same Erlenmeyer.

##### c. Titration

The distillate was titrated with HCl until a persistent color change from pink to colorless was observed. The volume of HCl used was recorded, and protein content was calculated from the nitrogen concentration.

##### 2.3.2.2. Fat content analysis (AOAC 2005) [18]

Fat content was determined using the Soxhlet extraction method with hexane as the solvent. The fat flask was dried in an oven for 30 minutes, cooled in a desiccator for 15 minutes, and weighed. The rice sample was weighed, wrapped in filter paper, and placed into the Soxhlet extractor with fat-free cotton. Extraction was carried out by refluxing with hexane for 5 hours until the solvent in the fat flask was clear. The solvent was then distilled off, and the flask containing the extracted fat was dried in an oven at 60 °C until a constant weight was achieved, cooled in a desiccator, and weighed. Fat content was calculated using the following formula:

$$\text{Fat Content (\%)} = \frac{B-A}{\text{Sample weight}} \times 100 \quad (1)$$

##### 2.3.2.3. Water content analysis (AOAC, 2005) [18]

Water content was determined using an oven-drying method. The empty cup was pre-dried at 100–105 °C for 30 minutes, cooled in a desiccator for 30 minutes, and weighed. A 500 g rice sample (B1) was placed in the cup and dried in an oven at 100–105 °C for 8–12 hours until a constant weight was achieved. The dried sample was cooled in a desiccator for 30 minutes and weighed (B2). Water content was calculated using the following formula:

$$\text{Water Content (\%)} = B1 - B2 \quad (2)$$

#### 2.3.2.4. Ash content analysis (AOAC, 2005) [18]

Ash content was determined by dry ashing. The empty crucible was pre-dried at 100–105 °C for 30 minutes until constant weight, cooled in a desiccator for 30 minutes, and weighed (B1). A 500 g rice sample was placed in the crucible, charred over a Bunsen burner, and then ashed in a furnace at 400 °C until gray. The temperature was then increased to 550 °C for 12 hours. The sample was cooled in a desiccator for 30 minutes and weighed (B2). Ash content was calculated using the following formula:

$$\text{Ash Content (\%)} = \frac{B2-B1}{\text{Sample weight}} \times 100\% \quad (3)$$

#### 2.3.2.5. Carbohydrate analysis using the by difference method [19]

Carbohydrate content analysis is carried out by subtracting 100% from water content, ash content, protein content, and fat content. Calculation of carbohydrate content can be done as follows:

$$\text{Carbohydrate (\%)} = 100\% - (\% \text{ash content} + \% \text{water} + \% \text{protein} + \% \text{fat}) \quad (4)$$

#### 2.3.2.6. Fiber content analysis (AOAC, 2005) [18]

A 2 g rice sample was placed in an Erlenmeyer flask, and 200 mL of boiling H<sub>2</sub>SO<sub>4</sub> solution (0.255 N) was added. The mixture was covered and boiled for 30 minutes, then filtered. The residue was washed with boiling water until free of acidity, returned to the flask, and boiled with 200 mL NaOH (0.313 N) for 30 minutes. After filtration, the residue was washed with boiling distilled water and 15 mL of 95% ethanol, then dried at 110 °C, ashed in a furnace at 500 °C, cooled in a desiccator, and weighed. The process was repeated three times until a constant weight was obtained. Crude fiber content was calculated using the following formula:

$$\text{Fiber Content (\%)} = \frac{\text{Fiber weight}}{\text{Sample weight}} \times 100 \quad (5)$$

#### 2.3.2.7. Analysis of vitamin A content (colorimetric method) [20]

Beta carotene measurement was carried out by extraction using ethanol with a colorimetric measurement procedure. The seeds were ground and defatted with hexane and extracted with 96% ethanol. The sample was ground until smooth and weighed as much as 0.6 grams, then put into a 40 ml tube. Then the sample was dissolved in acetone and added as much as 5 ml of 0.05% BHT in acetone, then 5 ml of 96% ethanol, and 10 ml of hexane solution. Next, the mixture was shaken at a speed of 180 rpm on ice or at a temperature of 4°C for 15 minutes, and 3 ml of ddH<sub>2</sub>O was added, then shaken for 5 minutes on ice. The homogeneous mixture was left at 4°C overnight. The top layer formed was then taken, and its absorbance was measured with a spectrophotometer at a wavelength of 520 nm.

#### 2.3.2.8. Analysis of anthocyanin content (Spectrometry - Vis method) [21]

Red rice yeast extract of 25 mg was diluted with 5.0 mL of acidified ethanol to pH 1.0. 1 mL of the extract solution was put into 2 vials, each added with 5 mL of KCl buffer pH 1.0 and 5 mL of Na-acetate buffer pH 4.5. Then shaken until dissolved, and the operating time is carried out for 30-60 minutes. After that, the absorbance value is calculated at the maximum wavelength and 700 nm. The calculation of the absorbance value of the sample solution (A) is as follows:

$$A = (A_{\text{vis}} - \text{max} - A_{700}) \text{ pH } 1,0 - (A_{\text{vis}} - \text{max} - A_{700}) \text{ pH } 4,5 \quad (6)$$

Total anthocyanin content (mg/100g):  $A \times MW \times DF \times V \times 1000 \epsilon \times I \times W$

where: A = Sample absorbance value; MV = Molecular weight of cyanidin-3-glucoside (449.2 g/mol); DF = Dilution factor; V = Volume of stock solution; W = Extract weight (g) 100 = Conversion factor for calculation in mg/100g sample;  $\epsilon$  = Molar absorptivity of cyanidin-3-glucoside = 26,900 L/ (mol.cm); I = Cuvette width = 1 cm

## 2.4. Data Analysis

Data analysis using the F test, if the gamma ray irradiation treatment on red rice cultivar Sidikalang has a significant effect, it is tested using the Tukey test.

### 3. Results and Discussion

The results of the data analysis showed that the gamma ray irradiation treatment had a significantly different effect on the biochemical content (fiber and vitamin A) compared to the treatment without irradiation (control), while on the biochemical content (protein, fat, ash, and carbohydrates), gamma ray irradiation treatment was not significantly different from the control. The results of data analysis showed that gamma ray irradiation treatment gave a significant difference in the bioactive content (anthocyanin) compared to that without gamma ray irradiation (control).

**Table 1.** Results of Biochemical and Bioactive Analysis of Rice Cultivar Sidikalang (M4) Seeds

Treatment	Biochemical and Bioactive Content							
	Water content	Protein	Fat	Fiber	Anthocyanin	Vitamin A	Ash	Carbohydrate
	%	%	%	%	mg/100g	µg	%	%
M4R0	9.02 <sup>ab</sup>	18.67 <sup>a</sup>	2.99 <sup>a</sup>	14.25 <sup>a</sup>	0.240 <sup>c</sup>	0.030 <sup>a</sup>	2.67 <sup>ab</sup>	29.35 <sup>a</sup>
M4R1	8.52 <sup>b</sup>	19.18 <sup>a</sup>	2.74 <sup>a</sup>	11.13 <sup>c</sup>	0.300 <sup>a</sup>	0.030 <sup>a</sup>	3.17 <sup>a</sup>	29.87 <sup>a</sup>
M4R2	9.60 <sup>a</sup>	20.88 <sup>a</sup>	2.69 <sup>a</sup>	13.12 <sup>b</sup>	0.227 <sup>c</sup>	0.023 <sup>a</sup>	1.83 <sup>b</sup>	31.31 <sup>a</sup>
M4R3	8.90 <sup>ab</sup>	19.83 <sup>a</sup>	3.54 <sup>a</sup>	11.70 <sup>c</sup>	0.300 <sup>ab</sup>	0.020 <sup>a</sup>	2.33 <sup>ab</sup>	30.06 <sup>a</sup>

Note: Numbers followed by different letters in the same column and row indicate significant differences according to Tukey's test at  $\alpha = 5\%$

The results of the analysis showed that the induction of gamma ray irradiation at a dose of 200-600 Gy on the water content of Sidikalang cultivar red rice did not provide a significant difference compared to without irradiation (0 Gy), but there was the highest water content at a dose of 400 Gy which was significantly different from the 200 Gy dose but not significantly different from the 600 Gy dose treatment. In line with the reported research results [22], gamma ray irradiation increased the water content of rice from various types of accessions and different varieties, but it was still <12%. Water content is an important component in food ingredients, it is assumed that water can affect the appearance, texture, and taste of food. Water content in food ingredients determines the acceptability, freshness and durability of the ingredients [23]. From the results of this study, the analysis of water content, although it increased due to the induction of gamma ray irradiation, the water content of the red rice cultivar Sidikalang was still below 13%, where water content is one of the main parameters in the quality standards of rice flour [24].



**Figure 1.** Red rice of cv Sidikalang (M4) seed induced by gamma ray irradiation R0, R1, R2, and R3

Figure 1 shows that the overall shape of the R0, R1, R2 seeds is almost the same, but in R3, there are differences in the seeds, where some of the R3 seeds resemble the R0, R1, R2 seeds, and some of the R3 seeds (smaller) and more yellow in color do not resemble R0, R1, and R2. The analysis results show that gamma irradiation tends to increase the protein content in the Sidikalang cultivar red rice. Although statistical testing has not shown a significant effect on the control, the analysis results show that the highest protein content is found at an irradiation dose of 400 Gy, which gives a protein content of 20.88%, while at a dose of 0 Gy, the protein content is 18.67%. This shows that the applied gamma-ray irradiation can increase protein content. In line with the research results reported [25], gamma ray irradiation increased the protein

content of 35 strains of rice Mentik susu compared to non-irradiated rice, which is in accordance with the opinion of [26], where gamma irradiation can induce the opening and denaturation of protein structures to produce new properties.

The analysis results show that the highest fiber content (14.25%) was found at 0 Gy, which was significantly different from 200 Gy (11.13%), 400 Gy (13.12%), and 600 Gy (11.70%), where irradiation induced a decrease in fiber content compared to non-irradiated. According to research [27], Exposure to ionizing gamma radiation in biological systems can trigger various chemical and physical reactions that cause biological damage [28], adding that free radicals produced by this radiation have the ability to alter plant cells, affecting chemical and biological processes that are important for the survival of the organism.

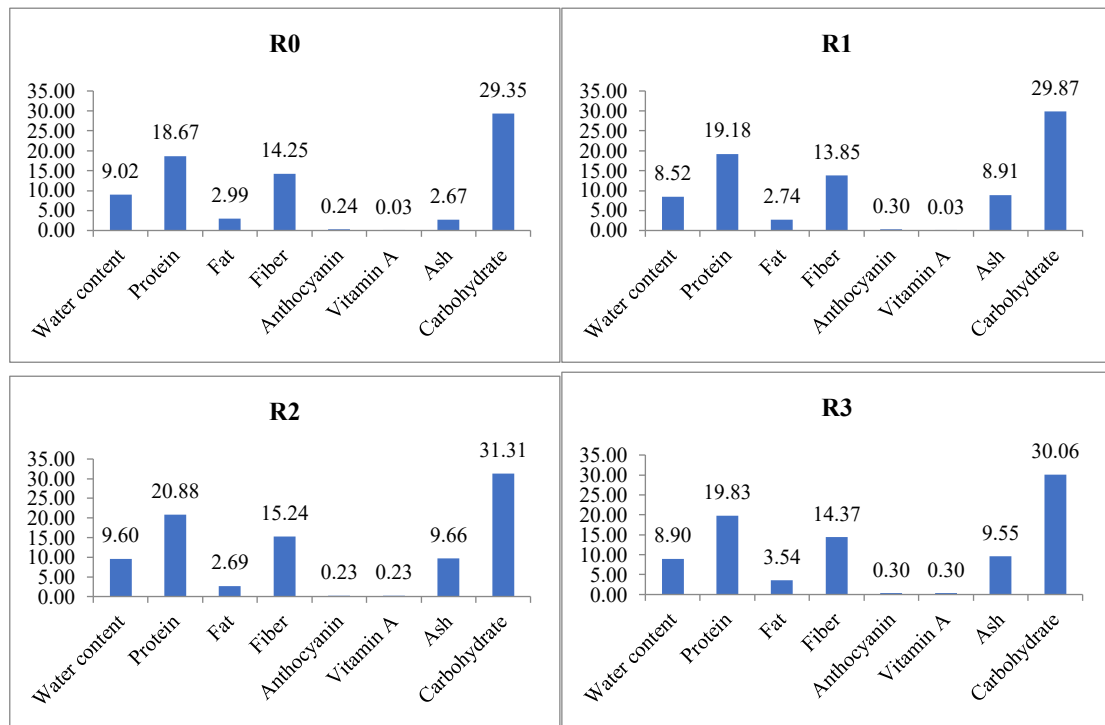
The results of the observation showed that gamma ray irradiation increased the anthocyanin content in red rice cv Sidikalang, where the doses of 200 and 600 Gy had the same value (0.030%), which was the highest compared to the doses of 200 Gy and 0 Gy. In line with this, an increase in anthocyanin content through gamma ray irradiation induction has been reported [29]. A radiation dose of 25 Gy has a higher anthocyanin content in flower petals compared to other treatments, where gamma ray radiation is able to damage growth cells, thus having an effect on the secondary metabolite compounds of red rosella plants.

The results showed that gamma-ray irradiation at a dose of 200 Gy resulted in a higher ash content (3.17%) compared to the control (2.67%). However, this increase was not statistically significant. Nevertheless, a tendency toward increased ash content was observed at the 200 Gy dose, while higher irradiation doses (400 and 600 Gy) tended to reduce ash content. Ash content serves as an indicator of the total mineral composition in a food material. In general, approximately 96% of most food components consist of organic matter and water, while the remaining fraction is composed of mineral elements. These mineral elements are commonly referred to as inorganic matter or ash content [23].

The results of the vitamin A content analysis test showed that gamma ray irradiation treatment did not provide any significant difference, where irradiation doses of 0 Gy and 200 Gy provided the same vitamin A content value (0.030 µg), and increasing the irradiation dose (400Gy-600Gy) tends to reduce vitamin A content. The results of the carbohydrate content analysis test showed that gamma ray irradiation increased the carbohydrate content, and tended to increase carbohydrate at a dose of 400 Gy (31.31%), but the results of the statistical test did not show any significant difference. According to [30], the effect of irradiation on gramineous plants can result in a decrease in carbohydrate synthesis, which is due to a decrease in enzyme activity due to ionization reactions with free radical molecules resulting from the excitation of water molecules.

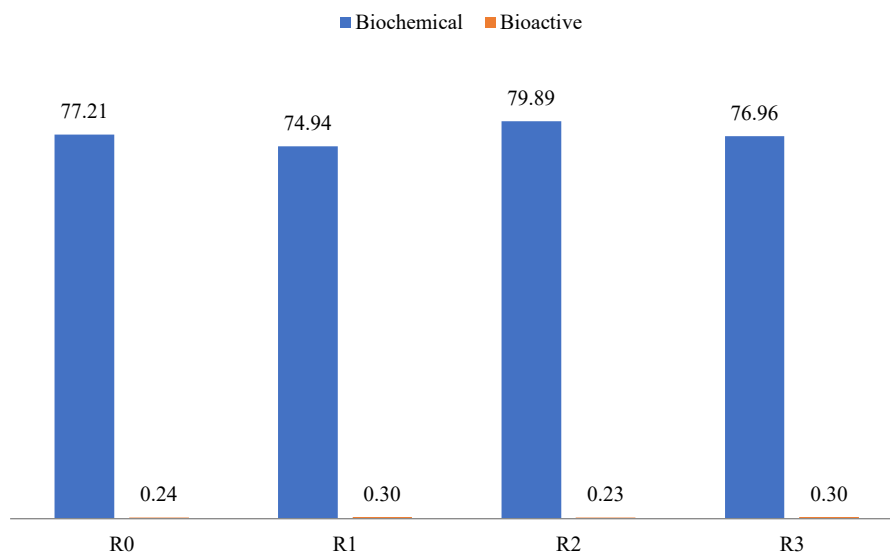
The test results showed that gamma ray irradiation did not provide a significant difference in the fat content of red rice cv Sidikalang, but the results of the fat analysis showed that the fat content at a dose of 600 Gy (3.54%) tended to increase compared to without irradiation (2.99%), a dose of 400 Gy (2.69), and a dose of 200 Gy (2.74). Fat is a component that is vulnerable to free radical attacks. Free radicals produced from gamma ray irradiation will interact with fat. As a result of this interaction, it will cause the fatty acid chain to break into compounds that are toxic to cells, such as various aldehydes (malondialdehyde), 9-hydroxy-nonenal, and various hydrocarbons such as ethane (C<sub>2</sub>H<sub>6</sub>) and pentane (C<sub>5</sub>H<sub>12</sub>). In addition, the interaction of free radicals can also result in cross-linking between two fatty acid chains or between fatty acids and peptide chains. This can cause serious damage to the cell membrane, which results in damage to the cells [31].

Although gamma-ray irradiation showed a tendency to increase protein and fat content in red rice cv. Sidikalang, the effect was not statistically significant compared to the control. This may be attributed to the fact that protein and fat contents in rice grains are largely determined by genetic background make less responsive to low to moderate levels of induced mutation. In addition, gamma-ray irradiation primarily affects DNA through random point mutations, which may not directly target genes involved in major protein or lipid biosynthesis pathways [32]. The use of the M4 generation, in which mutational effects may not yet be fully fixed, could also contribute to the absence of significant differences due to residual genetic segregation.



**Figure 2.** Effect of gamma ray irradiation on the biochemical and bioactive content of Sidikalang rice seeds

Figure 2 shows that gamma ray irradiation causes differences in biochemical content (water content, protein, fat, fiber, vitamin A, ash, and carbohydrate) and bioactive content of red rice cv Sidikalang. In Figure 2, it can be seen that gamma ray irradiation of 400 Gy provides the highest protein content compared to doses of 600 Gy, 200 Gy, and without irradiation (0 Gy). Furthermore, in the bioactive content of gamma ray irradiation, changes in the anthocyanin content of red rice cv Sidikalang irradiation doses of 200 Gy and 600 Gy provide the best anthocyanin levels compared to 400 Gy and without irradiation (0 Gy).



**Figure 3.** Comparison of the percentage of biochemical and bioactive content of red rice cultivar Sidikalang seeds induced by gamma ray irradiation

Figure 3. shows that the gamma irradiation dose provides the best percentage of biochemical content (water content, protein, fiber, and carbohydrate), namely at a dose of 400 Gy (79.46%) compared to doses of 0 Gy, 200 Gy, and 600 Gy. The best bioactive content (anthocyanin) was found at irradiation doses of 200 Gy and 600 Gy compared to irradiation doses of 0 Gy and 400 Gy.

#### 4. Conclusion

Gamma ray irradiation causes significant changes to the biochemical and bioactive content of Sidikalang cultivar red rice (water content, fiber, ash, and anthocyanin). Gamma ray irradiation at a dose of 400 Gy provided the highest biochemical content (water content, protein, fiber, and carbohydrate) compared to other doses, while the highest bioactive content value of local red rice cultivar Sidikalang was found at doses of 200 Gy and 600 Gy. This research should be continued up to the M8 generation (to be stable) considering that gamma ray irradiation induction can increase several biochemical and bioactive contents of red rice cultivar Sidikalang.

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