Starch, amylose and amylopectin levels of M5 and M6 generations of black rice irradiated by gamma Co\textsuperscript{60} ray

R P Bachtari, S Listyawati and Sutarno
Department of Biology, Faculty of Mathematics and Natural Sciences Sebelas Maret University, Surakarta, Indonesia
E-mail: shantilistyawati@staff.uns.ac.id

Abstract. M5 and M6 of black rice generation that were the product of radiation Cobalt-60 on 300 Gy showed phenotype changed, one of which was the improvement of nutrition content. A polysaccharide is a kind of black rice nutrition from amylose and amylopectin polymers. This research's purpose was to know the amount of starch, amylose, and amylopectin from M5, M6, and non radiated black rice. Content of starch was measured by the Luff Schoorl method, amylose by the Iodine-Colorimetric method, and amylopectin levels were obtained from the difference between starch and amylose content. The results showed that the starch content of M5 and M6 lower than non-radiation, i.e., M5 was 73.77%, M6 was 62.20%, and non-radiated was 83.28%. The amylose in irradiated black rice was 71.08% (M5) and 59.33% (M6), which was lower than non-irradiated (4.85%). Irradiated black rice has lower amylopectin levels than to non-irradiated black rice. Non-radiation black rice was 8.43%, M5 was 2.69%, and M6 was 2.86%.

1. Introduction
Rice is a staple food for the people because it contains a variety of nutrients, one of which is carbohydrate. Carbohydrates in rice are a source of energy for the body, 100 grams of rice will produce 360 kcal\textsuperscript{[14]}. Rice has various types, i.e., white rice, brown rice, chocolate rice, and black rice. They contain different nutrients and active compound each other. One of the rice which is currently becoming popular is black rice. This rice was consumed as a functional food because it contains active compounds that are good for body health. Black rice is Indonesian’s local rice. Various types of cultivars from black rice can be found around the Special Region of Yogyakarta. In general, black rice has the characteristics of high plant posture, brown rice grain, the color of endosperm is black and a long harvest period. The black color of rice (endosperm) is due to the presence of anthocyanin in the aleuron and pericarp that is controlled by gene\textsuperscript{[8]}.

Black rice has several benefits for the body because the content of chemical compounds can help to control the body's metabolism and homeostasis. The disadvantage of black rice is the long harvest time. The length of the harvest period encourages breeding by irradiation on black rice, which is a collaboration between BATAN and Sebelas Maret University. From the results of the study, the irradiated black rice has entered the 5th generation or (M6) 6th mutant. The research produced black rice with a short harvest period and shorter plant height. However, black rice M5 has lowered anthocyanin, which causes black rice looked semi-black\textsuperscript{[11]}. In addition, the results obtained are short
harvest periods and productive tiller\textsuperscript{[16]}. Black rice that has undergone breeding also has improved nutritional content, including fat, carbohydrates, protein, mineral, and water content\textsuperscript{[5]}. Besides the anthocyanin compounds, amylose and amylopectin can also be found in this black rice. Amylose and amylopectin compounds are starch-forming glucose polymers; these compounds determine the texture of rice fluffiness. Based on the amylose content, rice can be divided into four groups, i.e. very low amylose content (<10%), low amylose content (10% -20%), moderate amylose content (20% -24%), and high amylose content (> 25%). The lower the amylose content in rice, the fluffier texture of the rice, while the fluffiness of rice will decrease along with the high level of amylose in rice. This is in contrast to the amylopectin content in rice\textsuperscript{[15]}. Amylose is composed of a long and unbranched $d$-glucose residual chain; it connected by $(\alpha1\rightarrow4)$. The chain varied based on the molecules' weight and shape. The amount of its molecule range from a few thousand until more than a million. On the other hand, amylopectin has higher molecule weight and a lot of branches than amylose. The glycosidic chain that connects between glucose residues on the amylopectin chain is $(\alpha1\rightarrow4)$, for branching point that occurs every 24 – 30 residue, connected by $(\alpha1\rightarrow6)$\textsuperscript{[12]}. M5 and M6 black rice is the result of breeding using Gamma Co$^{60}$ rays. The breeding carried out produced black rice with morphological and nutritional improvements. One of the nutrients that can be improved due to breeding is the content of amylose and amylopectin, which play a role in determining the fluffiness of black rice, so this study aims to determine the amylose and amylopectin levels of black rice M5 and M6 irradiated with Gamma Co$^{60}$ rays.

2. Research method

The study was conducted in March to April 2019 at the Integrated Mathematics and Natural Sciences Laboratory (Lab. MIPA Terpadu) and the Biology Laboratory of the Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Surakarta.

2.1 Materials

The materials used in this study are non-irradiated black rice (\textit{Oryza sativa} L.), fourth-generation (M5) black rice, and fifth-generation (M6). The fourth and fifth-generation black rice is the result of the breeding of \textit{Cempo Ireng} cultivar rice using irradiation of Gamma Co$^{60}$ rays in collaboration with BATAN-UNS. The M5 black rice as the test material was taken in Gatak, Tempel, Sukoharjo, and the M6 black rice was taken in Klaten, Central Java.

2.2 Equipment

The tools used to test starch, amylose, and amylopectin levels are glassware, hot plate stirrers, Kjeldahl flasks, titration tools, reflux tools, analytical balance, and UV-Vis spectrophotometers.

2.3 Working procedure

2.3.1 Starch content test (\textit{SNI: 3451: 2011}). Testing of starch content using the Luff Schoorl method. This method uses the titration technique. A sample of 1 gram black rice (flour) was put in a 100 ml Erlenmeyer and added with 3% HCl of 40 ml. The mixture is refluxed using a back coolant for 3 hours. After refluxing, the solution is cooled and neutralized using 30% NaOH and added with 3% CH$_3$COOH. The solution was put in a 100 ml volumetric flask and added with the aqua dest ad. The solution is filtered, and 10 ml of filtrate is taken and then put in an Erlenmeyer. The filtrate was added with 25 ml of Luff Schoorl solution and 15 ml of aqua dest. The mixture is heated till boiling. The cooled solution is then added with 15 ml of 20% KI and 25 ml of 25% H$_2$SO$_4$. The solution is titrated using 0.1 N Na$_2$S$_2$O$_3$ until the solution turns pale yellow. 1% of starch is added as much as 2 ml until the solution turns purplish-blue. Titration continues until the purplish-blue disappears. The amount of 0.1 N Na$_2$S$_2$O$_3$ was calculated. Blank solution testing is done by replacing the filtrate with distilled
water as much as 25 ml. Starch content was measured 3 times. Calculation of starch content can be done using the following formula:

\[
\text{Starch Level} = 0.9 \times \text{Glucose level}
\]

\[
\text{Glucose Level} = \frac{w \times f_p}{w_1} \times 100\%
\]

Note:
- \(w\) = sample weight (mg)
- \(w_1\) = glucose weight based on table 2 (mg). The amount of Na\(_2\)S\(_2\)O\(_3\) in the table is sought by reducing the volume of the blank titer with the volume of the sample titer.
- \(f_p\) = dilution factor (0.01)

2.3.2 Amylose and amylopectin content test\(^7\). An amylose content test was carried out using the Iodin-Colorimetric method. 100 mg black rice flour was put into 100 mL volumetric flask and then given 1 mL of 95% ethanol and 9 mL NaOH 1 N. The solution is heated in a 100°C water bath for 10 minutes and cooled for 1 hour. The solution is diluted with distilled water up to 100 mL. After dilution, the solution is pipetted as much as 5 mL and put into a 100 mL volumetric flask. The solution was then added 1 mL of 1 N acetic acid and 2 mL of 2% I\(_2\) and diluted to a volume of 100 mL. The solution was shaken and allowed to stand for 20 minutes; then the absorbance was measured three times with a spectrophotometer \(\lambda\) 645 nm. Amylose levels are calculated by the formula:

\[
\text{Amylose level} (\%) = \frac{[\text{amilosa}] \times f_p \times 100}{W} \times 100
\]

Note:
- \([\text{amylose}]\) = amylose concentration
- \(f_p\) = dilution factor (0.02)
- \(W\) = sampel weight (mg)

Amylopectin levels can be obtained using the following formula\(^6\):

\[
\text{Amylopectin levels} (\%) = \text{Starch levels} (\%) - \text{Amylose levels} (\%)
\]

3. Results and discussion

The test results of starch, amylose, and amylopectin levels in non-radiation black rice, black rice M5, and M6 can be seen in Table 1. The results show that there are differences in starch content, amylose, and amylopectin levels. The levels of starch on non-radiation black rice were 83.28%, and the levels of black rice M5 and M6 were 73.77% and 62.20%. Non-radiation black rice amylose content was 74.85%, M5 was 71.08%, and M6 was 59.33%. While the amylopectin content in non-irradiated black rice was 8.43%, M5 was 2.69%, and in M6 black rice was 2.86%. The instability of the nutritional content in the form of amylose and amylopectin in black rice M5 and M6 irradiation may due to the instability of genes that control the starch content in black rice grains. According to\(^{13}\), mutations can bring better changes in genetic traits. Gene mutations due to gamma rays given to a plant can be irreversible, which means that traits passed down to the next generation can return to normal (original characters).

Table 1. Starch, amylose, and amylopectin levels of black rice.

| Samples            | Starch Level (%) | Amylose Level (%) | Amylopectin Level (%) |
|--------------------|-----------------|------------------|-----------------------|
| Non-Radiation Black Rice | 83.28           | 74.85            | 8.43                  |
| M5 Black Rice      | 73.77           | 71.08            | 2.69                  |
According to \cite{4} and \cite{1} starch synthesizing genes play a role in determining the amylose, amylopectin content and physico-chemical properties of rice. The gene that plays a role in determining the formation of amylose and amylopectin is the Wx gene. The gene produces an enzyme that can synthesize amylose and amylopectin, namely Granule-bond Starch Synthase (GBSS), Starch Branching enzyme (SBE), debranching starch enzyme, and Starch Synthase (SS). The four enzymes above play a role in synthesizing amylose and amylopectin in rice. The role of the Wx gene that produces these enzymes also determines the structure and amount of amylose and amylopectin. According to \cite{10}, gamma rays can cause the mutation of the Wx gene, so that it can affect amylose and amylopectin levels in rice.

Based on the amylose content, rice can be divided into four groups, namely sticky rice or rice with very low amylose content (<10%), rice with low amylose content (10% -20%), rice with moderate amylose content (20% -24%) and rice with high amylose content (> 25%)[13]. Overall non-radiation black rice, M5, and M6 are included in rice with high amylose content. From the measurement results of amylose and amylopectin levels that have been carried out, it shows that the amylose content of non-irradiated black rice is greater than that of M5 and M6 black rice, while the amylose level of M6 is smaller when compared to M5 black rice. According to \cite{10}, high levels of amylose in black rice affect the texture of rice, namely becomes less fluffy (drier). From the results obtained, the texture of rice from M6 black rice is fluffier than M5 and Non-Radiation black rice.

High levels of amylose in non-irradiated black rice, M5, and M6 cause the structure of rice when cooked to become not fluffy. This condition, according to \cite{12} and \cite{3}, due to the chemical chain of amylose in the form of long, unbranched β-glucose residues. The chain is connected by connections (α1 – 4). The existence of these unbranched chains makes it difficult for amylose to bind to other molecules and difficult to hydrolyze.

Amylose and amylopectin levels have related to the glycemic index in rice. Amylose amylopectin levels can be used to predict the level of GI (Glycemic Index) in black rice. High levels of amylose indicate that rice has a low GI. This is because high levels of amylose make rice less susceptible to gelatinization so it is not easily hydrolyzed into glucose. Low glycemic index shows that rice can slow down the release of hydrolysis (glucose) results in the body. Rice, with a low glycemic index (GI) is very suitable for consumption for diabetics\cite{2}. High levels of amylose in non-radiation black rice, M5, and M6 is one of the markers that the black rice has a low GI, so it can slow down the process of glucose release associated with postprandial blood glucose levels.

### 4. Conclusion

The results of black rice breeding with Co\textsuperscript{60} gamma-ray radiation showed different levels of starch, amylose, and amylopectin levels between M5 and M6 generations. These generations showed a decrease in starch and amylose content. The levels of starch M5 and M6 were lower than non-radiation black rice, with the value of non-radiation black rice starch being 83.28%; M5 of 73.77%; and M6 was 62.20%. Amylose levels in irradiated black rice decreased, with non-radiation black rice amylose values that were 74.85%, M5 at 71.08%, and M6 at 59.33%. While the amylopectin content in non-irradiated black rice was 8.43%, M5 was 2.69%, and in M6 black rice was 2.86%.

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