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Effect of Moringa Leaves and Jackfruit Seeds as Rice Substitute to Reduce Blood Glucose Levels in Mice

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Abstract

Most Indonesians consume white rice, which has a high Glycemic Index (GI). Eating foods with high GI can lead to the occurrence of Diabetes Mellitus (DM). DM is a metabolic disorder characterized by chronic hyperglycemia. Alternative solutions are needed to overcome diabetes mellitus, one of which is by using rice substitute which has a low Glycemic Index (GI). Moringa leaves (*Moringa oleifera*) and jackfruit seeds (*Artocarpus heterophyllus* lam.) contain flavonoids that have the potential to be used as ingredients for making rice substitute. Flavonoids function to increase antioxidant enzymes, neutralize free radicals, lower blood sugar, overcome fatigue, and protect insulin-producing pancreatic ß cells. This study aims to analyze the content, physical and chemical characteristics, and antidiabetic activity of innovative rice substitute. In this study, an rice substitute formulation was made from Moringa flour: jackfruit flour in the ratio of 3:1 (P1), 1:1 (P2), and 1:3 (P3). Analog rice was tested on mice which were divided into five groups, namely positive control, negative control, and a combination of extracts P1, P2, and P3. Data analysis was carried out using SPSS 25 software. The results obtained showed that the rice substitute formulations P1, P2, and P3 were able to reduce blood sugar in mice, but P2 best reduced blood sugar, which was 11 mg/dL and 22 mg/dL.

Keywords: Antidiabetes; Analog Rice; Jackfruit Seeds; Moringa Leaves; Diabetes Mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that affects 573 million adults worldwide, and this number is projected to rise to 643 million by 2030 and 783 million by 2045 (Federation, 2024). In Indonesia, in 2020, 6.2% of the population, or approximately 10.8 million people, were recorded as having diabetes, and this number is predicted to continue increasing each year (Yusransyah et al., 2022). In Bali Province, type 2 diabetes ranks second among the top ten most common diseases in outpatient and inpatient hospital settings, with 60,423 cases in 2019 (Pratama & Ratnasari, 2021). This increase in prevalence is concerning because diabetes not only causes long-term metabolic disorders but also leads to severe complications and a decline in patients' quality of life.

One of the key factors contributing to the high incidence of diabetes is the consumption of high-glycemic index foods, particularly white rice, which remains the staple food for the majority of the Indonesian population. According to Soviana & Pawestri (2020), the glycemic index of white rice reaches 128% per 300 grams per day, placing it in the high-glycemic category. In response to this issue, various rice substitute innovations have begun to be developed using alternative ingredients with greater health potential. One such example is rice substitute made from porang tubers. However, the antioxidant content in porang is relatively low, so its effectiveness in controlling blood glucose levels still needs to be improved (Februyani & Zuhriyah, 2022).

Considering the need for healthier functional carbohydrate sources, innovations in analog rice made from locally sourced foods rich in bioactive compounds offer a promising alternative. Moringa leaves (Moringa oleifera) and jackfruit seeds (Artocarpus heterophyllus Lam.) are two locally available, affordable, and phytochemical-rich ingredients. Moringa leaves contain quercetin, a flavonoid with strong antioxidant activity that has been shown to enhance insulin sensitivity through activation of the AMPK and GLUT4 pathways (Priyanto et al., 2023). On the other hand, jackfruit seeds contain complex carbohydrates, resistant starch, dietary fiber, and flavonoids that synergistically slow glucose absorption in the intestines and maintain stable blood glucose levels (Dwitiyanti et al., 2019).

Therefore, this study aims to develop an rice substitute product from a combination of moringa leaves and jackfruit seeds and analyze its active compound content, physical and chemical characteristics, and antidiabetic activity through in vivo testing on experimental animals. This research is expected to contribute to the development of local functional foods that support the prevention and management of diabetes mellitus in a more comprehensive and sustainable manner.

MATERIALS AND METHODS

Place and Time of Research

This research was conducted at the Research Laboratory and Animal Laboratory of the Faculty of Medicine and Health Sciences, Warmadewa University, over a period of four months from April to June 2024.

Tools and Materials

This study used several tools and materials. The tools used were a knife, 1 ml syringe, pot, container, oven, grinder, mortar, strainer, beaker, measuring cup, sieve, blender, cutting board, digital scale, oven, mouse probe, scissors, aluminum foil, baking paper, glucodr, and blood sugar strips. Meanwhile, the materials used include mice, NaCl, distilled water, moringa leaves, jackfruit seeds, alloxan, and Carboxy Methyl Cellulose (CMC).

Population and Sample

The population in this study consisted of healthy male Mus Musculus mice, aged approximately 40 days, weighing ±25 grams. The study sample consisted of 25 male mice selected randomly and meeting the inclusion and exclusion criteria. The sample size was determined based on Federer's formula.

Research Variables

This study has three variables, namely independent variables, dependent variables, and control variables. The independent variables used in this study are the administration of moringa leaf flour and jackfruit seed flour, respectively. The dependent variable used in this study is blood sugar levels in mice injected with alloxan. The control variables used in this study are age, body weight of the mice, sex of the mice, mouse care, duration of treatment, and type of treatment.

Research Ethics

This study has been approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Warmadewa University, with protocol number **430.15.05.24**. All procedures involving laboratory animals were conducted in accordance with the principles of animal welfare and followed the ethical guidelines for laboratory animal research. Mice were treated humanely to minimize pain and stress.

Research Procedures

Production of moringa leaf flour

Sort, wash, and drain 200 g of fresh moringa leaves before processing. Then, oven dry the moringa leaves at 50 °C for 1 hour. Next, blend the dried moringa leaves and sift them using a 80 mesh flour sifter (Faturochman et al., 2022).

Production of jackfruit seed flour

Jackfruit seeds are sorted, washed, and boiled at 70°C for 30 minutes to soften them and reduce their odor. They are then sliced and dried in an oven at 60°C for 2 hours. Next, they are ground in a blender and filtered using an 80-mesh sieve (Cicilia et al., 2021).

Production of analog rice

The production of rice substitute begins with weighing moringa leaf flour and jackfruit seed flour according to the treatment ratio. Then, CMC is weighed at 5% of the total flour weight. After measurement, the ingredients are mixed with 80 mL of distilled water. The mixture is stirred evenly and slowly until well combined. Once mixed, the dough is shaped using a noodle maker and dried in an oven at 70°C for 2 hours (Kumolontang & Edam, 2019).

Tabel 1. Comparison of Analog Rice Ingredients.

Treatment	Moringa Leaf Flour (gr)	Jackfruit Seed Flour (gr)
P1	25	75
P2	50	50
P3	75	25

Organoleptic Testing Procedures

This test was conducted using a survey. Organoleptic testing was carried out on several parameters, namely color, aroma, and texture (Setiawan et al., 2022).

Antioxidant Level Testing Procedures

The DPPH antioxidant test was conducted by mixing 2 mL of 40 ppm DPPH solution and 2 mL of test solution, then mixing them with a vortex. Absorption was measured using a UV-Vis spectrophotometer, and each concentration was recorded (Februyani & Zuhriyah, 2022).

Phytochemical Testing Procedures

Identification of secondary metabolites in the form of flavonoids, tannins, saponins, and alkaloids using phytochemical testing methods in accordance with the methods described in the journal by Yulia et al. (2022).

Animal Testing Procedures

Mice were fasted for 8 hours (only drinking water was provided) and then induced with an injection of alloxan monohydrate 140 mg/kgBW. On the third day, after the alloxan injection, blood sugar was taken to ensure the success of alloxan in inducing diabetes in mice. The mice were divided into 5 groups: group 1 without treatment, group 2 given metformin at 10 mg/kg body weight, and groups 3 to 5 given standard feed mixed with analog rice (P1, P2, P3) at a ratio of 1:5. Feeding was administered at 11:00 AM via a feeding tube and provided in the cage. Blood glucose levels were measured on day 7 and day 10 post-treatment following the administration of analog rice (Priyanto et al., 2023).

In Silico Testing Procedures

This study used an in silico method consisting of ADME prediction and molecular docking. ADME testing was performed using the Swiss ADME website. Molecular docking testing uses the 3D structure of the compound obtained from PubChem, while the target protein structure is taken from the Protein Data Bank (PDB). Docking is performed using AutoDock Vina to obtain binding affinity values. Affinity values ≤ -6.0 kcal/mol are considered to indicate strong binding. Interactions are visualized using PyMOL (Goenharto et al., 2020; Kour et al., 2020).

Data Analysis

Data from in vivo testing were analyzed using SPSS 25.0. Normality testing was performed using Shapiro-Wilk, and differences between variables were analyzed using One Way ANOVA at a significance level of 95% (α = 5%). If there were significant differences, a Post Hoc Bonferroni test was performed (Faturochman et al., 2022).

RESULTS AND DISCUSSION

Result-1 Characteristics of Moringa Leaf and Powder and Jackfruit Seed Powder

The moringa leaf flour obtained appears to have a striking deep green color. This color indicates that the drying and grinding process successfully preserved the natural pigment of moringa leaves, namely chlorophyll. The texture appears very smooth and uniform, indicating that the milling process was carried out optimally. In addition, the aroma is still characteristic of dried leaves, indicating that the volatile compounds and active components of the moringa leaves are largely preserved. Meanwhile, jackfruit seed flour has a lighter color, a creamy brown hue, which is the natural color of the dried jackfruit seed endosperm. The texture of jackfruit seed flour is also smooth and does not clump, indicating that its moisture content is low and the drying and grinding processes were carried out effectively. Both types of flour exhibit physical characteristics suitable for use as raw materials in the formulation of functional food products or nutraceutical products.

Figure 1. Picture of Moringa Leaf and Jackfruit Seed Flour.

The phytochemical test results presented in Table 2 show that moringa leaf powder contains several bioactive secondary metabolites, namely flavonoids, tannins, phenols, and saponins. The presence of flavonoids is indicated by a color change in the solution to orange, while tannins produce a greenish-black color reaction. The test for phenols shows a blue-black color change, and the presence of saponins is confirmed by the formation of foam in the test solution. Conversely, alkaloids, steroids, and terpenoids were not detected because no characteristic color changes or precipitates occurred during the testing.

Meanwhile, phytochemical test results for jackfruit seed flour Table 3 showed the presence of tannins, phenols, and saponins, indicated by greenish-black and bluish-black color changes for tannins and phenols, as well as the formation of foam in the saponin test. However, alkaloids, flavonoids, steroids, and terpenoids were not detected in this sample as they did not exhibit reactions indicating the presence of these compounds.

Table 2. Phytochemical Test Results of Moringa Leaf.

No	Types of Tests	Result	Description
1	Alkaloid	Negative	No sediments
2	Flavonoid	Positive	There is a change in color to orange
3	Tanin	Positive	There is a change in color to dark green
4	Fenol	Positive	There is a change in color to dark blue
5	Steroid	Negative	No changes in purple, blue, and green colors
6	Terpenoid	Negative	There is no color change to brown
7	Saponin	Positive	There is foam

Table 3. Phytochemical Test Results of Jackfruit Seeds.

No	Types of Tests	Result	Description
1	Alkaloid	Negative	No sediments
2	Flavonoid	Negative	There is no color change to orange
3	Tanin	Positive	There is a change in color to dark green
4	Fenol	Positive	There is a change in color to dark blue
5	Steroid	Negative	No changes in purple, blue, and green colors
6	Terpenoid	Negative	There is no color change to brown
7	Saponin	Positive	There is foam

The results of the antioxidant activity test shown in Table 4 indicate that moringa leaf powder has an IC_{50} value of 1749.29 ppm, while jackfruit seed powder has an IC_{50} value of 5402.62 ppm. The IC_{50} value stands for Inhibitory Concentration 50%, which is the concentration of a substance required to inhibit 50% of free radical activity in an antioxidant test (Nur Danastry et al., 2021). In practice, as in the DPPH free radical test, the IC_{50} value reflects how strong the antioxidant capacity of a sample is at lower concentrations to achieve 50% inhibition, indicating higher antioxidant activity (Situmeang et al., 2023). In other words, the smaller the IC50 value, the stronger the antioxidant activity of the sample, as only a small concentration is required to achieve a significant radical-scavenging effect. Based on the data, moringa leaf powder has significantly higher antioxidant activity compared to jackfruit seed powder. The differences in phytochemical test results in Tables 2 and 3 show that moringa leaf powder contains bioactive compounds such as flavonoids, tannins, phenols, and saponins, which are known to play a role in free radical scavenging mechanisms. Conversely, jackfruit seed flour does not show the presence of flavonoids, which are one of the most dominant groups of phenolic compounds in antioxidant activity. Therefore, the low antioxidant activity in jackfruit seed flour is likely due to the limited types and amounts of antioxidant-active compounds within it.

Table 4. Antioxidant Test Results.

No	Sample Type	Antioxidant Activity (IC50) ppm
1	Moringa Leaf Flour	1749,29
2	Jackfruit Seed Flour	5402,62

Result-2 Characteristics of rice substitute

Analog rice is an innovation derived from moringa leaves and jackfruit seeds as an antidiabetic agent. As shown in Figure 4.2, the analog rice innovation has a dark green color from the moringa leaves. In terms of aroma, P1, P2, and P3 all have the characteristic scent of moringa leaves. Regarding texture, P1 has a denser texture compared to P2 and P3. Meanwhile, P2 and P3 have a slightly softer texture.

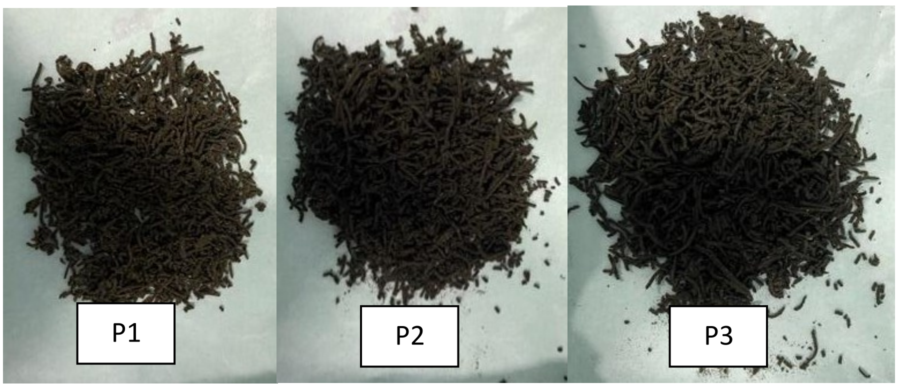

Figure 2. Analog Rice.

The high levels of carbohydrates and fats are directly proportional to the number of calories. This is because calories, as a unit of energy, come from the three main macronutrients: carbohydrates, proteins, and fats. Among the three, fats produce the highest amount of energy, at 9 calories per gram, while proteins and carbohydrates each produce 4 calories per gram (Handayasari et al., 2023). The high carbohydrate content in jackfruit seed flour is more dominant than moringa leaf flour, which has a higher protein and fat content.

Table 5. Test Results of Chemical Characteristics of Analog Rice.

Sample	Water (%bb)	Ash (%bb)	Protein (%bb)	Fat (%bb)	Carbohydrate (%bb)	Calories (%bb)
P1	5,308	5,902	2,281	1,236	85,27	361,328
P2	6,035	4,854	3,083	2,645	83,38	369,657
P3	3,097	3,159	5,346	4,769	83,63	398,825

Result-3 In Vivo Antidiabetic Testing

The in vivo testing of mice had 25 antidiabetic parameters divided into five groups. Each group consisted of five mice.

Table 6. Blood Sugar Level of Mice in In Vivo Testing (mg/dL).

Sample	H0	H7	H10
K +	147,6 ± 19,6	151,8 ± 19,0	136 ± 16,3
K -	149,2 ± 42,3	165,6 ± 14,1	155,2 ± 19,9
P1	124,6 ± 8,3	123,4 ± 10,2	100,4 ± 11,3
P2	138 ± 6,3	122,8 ± 23,0	107,2 ± 12,6
P3	127,2 ± 25,8	128,8 ± 10,9	116,6 ± 10,0

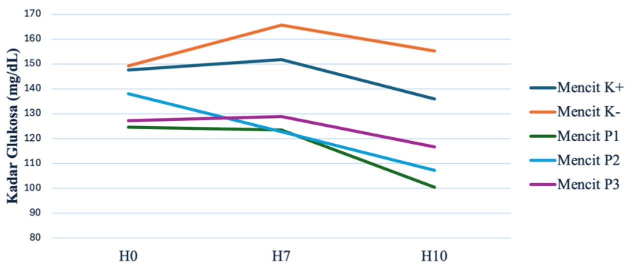

Figure 3. Graph of Changes in Blood Glucose Levels of Mice in Testing.

Based on Figure 3, there was a significant decrease in blood glucose levels in the treatment groups (P1–P3) on days 7 to 10, which was thought to be influenced by the active compounds in each ingredient. In contrast, the positive control group (K+) showed an insignificant decrease, while the negative control (K−) experienced an insignificant increase in blood glucose levels.

Based on phytochemical tests, rice made from moringa leaf flour and jackfruit seed flour contains active compounds such as tannins, saponins, flavonoids, and phenolics, which act as antidiabetics through various mechanisms. Tannins enhance glycogenesis and inhibit glucose absorption in the intestines. Saponins aid in pancreatic regeneration and increase insulin secretion. Flavonoids and phenolics function as antioxidants that protect pancreatic β-cells from oxidative stress and enhance glucose absorption (Meldawati et al., 2024 ; Wenas et al., 2020). Quercetin in moringa leaves, as one of the flavonoid derivatives, works by inhibiting the glucosidase enzyme and activating AMPK, which plays a role in various receptors regulating blood glucose levels (Selawati et al., 2024).

Result-4 In Silico Testing

Molecular docking results show that quercetin has a binding affinity value of -7.5 kcal/mol for the Glucose Transporter 4 (GLUT4) receptor. A more negative binding affinity value reflects a stronger binding affinity between the ligand and the receptor. Based on general criteria, an affinity < -6.0 kcal/mol is considered to have strong biological potential.

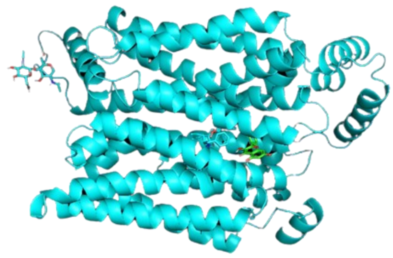
Figure 4. GLUT4 (Glucose Transporter 4) Receptor with Quercetin.

Quercetin (QE) plays a role in enhancing cellular glucose uptake through a mechanism involving GLUT4 (Glucose Transporter Type 4), particularly in skeletal muscle and adipose tissue. In diabetic patients, GLUT4 translocation to the plasma membrane is impaired due to insulin signaling abnormalities. QE can overcome this barrier by activating AMPK (AMP-activated protein kinase), thereby stimulating GLUT4 translocation independently of insulin. Additionally, QE increases GLUT4 expression and activates the PI3K/Akt and AMPK/Akt signaling pathways, further enhancing glucose uptake. This effect is reinforced by increased expression of the estrogen-α receptor, which supports further GLUT4 activation. Through these mechanisms, QE demonstrates therapeutic potential similar to rosiglitazone as a PPARγ agonist in diabetes management (Bule et al., 2019 ; Shi et al., 2019).

Result-5 Normality Test

Before conducting the One Way ANOVA test, the data was first tested for normality using the Shapiro-Wilk test because the number of samples in this study was less than 50. The results of the normality test can be seen in Table 4.6 below.

Table 7. Shapiro Wilk Normality Test.

Data Testing	Shapiro-Wilk		
	Statistic	df	Sig.
Decrease in Blood Glucose Levels	0.988	75	0.692

The normality test shows that a significance value of $p > 0.05$ means that the data is normally distributed. The normality test results show $p = 0.692$, so the data is considered normal and can be analyzed using One Way ANOVA.

Result-6 Homogeneity Test

The results of the variance homogeneity test can be seen in Table 8

Table 8. Homogeneity Test of Blood Glucose Levels.

Data Testing	Levene Statistic	df1	df2	Sig.
Decrease in Blood Glucose Levels	0.807	4	70	0.525

The test of variance homogeneity showed a p-value > 0.05, which means that there was no difference in variance between data groups, so the data variance could be considered homogeneous. Since the conditions of normality and homogeneity were met, the analysis could be continued with a one-way ANOVA test to determine the effect of feed variation on blood glucose levels.

Result-7 One Way ANOVA Test

Multivariate analysis of variance is a method for testing differences in variance. In ANOVA, the variances being compared come from a single interrelated variable. This study aims to analyze the effect of feed variation on the decrease in blood glucose levels in mice. The hypotheses proposed in this test are as follows.

H_0 : Variations in analog rice feed had no effect on glucose levels in mice.

H_1 : Variations in analog rice feed affect blood glucose levels in mice.

The results of the ANOVA analysis for blood glucose levels are shown in Table 4.8 below.

Table 9. One Way ANOVA Test Analysis Results.

Data Testing	df	MeanSquare	F	Sig.
Decrease in Blood Glucose Levels	4	4410.087	9.486	0.000

The benchmark for decision making in ANOVA testing is based on the significance value. If the significance value (p) < 0.05, then H_0 is rejected and $H_1$is accepted. In this analysis, a significance value of 0.000 was obtained for blood glucose levels, so H_0 is rejected and $H_1$is accepted. Thus, it can be concluded that variations in analog rice feed significantly affect the decrease in blood glucose levels in mice.

Result-8 Post Hoc Bonferroni Test

To determine the significant difference between blood glucose levels in the treatment group and the control group, a post-ANOVA (Post Hoc) test was conducted using the Bonferroni method, considering that the data analyzed was homogeneous. If the significance value in the Post Hoc test (p) < 0.05, it can be concluded that there is a significant difference between the treatment group and the control group. The results of the Bonferroni Post Hoc test related to the decrease in blood glucose and uric acid levels in mice are presented in Table 4.9 below.

Table 10. Results of Bonferroni Post Hoc test in One Way ANOVA.

Data Testing	Sig.	K+	K-	P1	P2	P3
Decrease in Blood Glucose Levels	K+	-	1.000	0.004*	0.057	0.097
	K-	1.000	-	0.000*	0.001*	0.001*
	P1	0.004*	0.000*	-	1.000	1.000
	P2	0.057	0.001*	1.000	-	1.000
	P3	0.097	0.001*	1.000	1.000	-

The difference in blood glucose levels between the treatment and control groups was tested using Post Hoc Bonferroni. If the significance value (p) < 0.05, it means that there is a significant difference between the groups. The * sign indicates p < 0.05, indicating a difference between groups. Based on the results of the Post Hoc Bonferroni analysis of blood glucose levels, a significant difference was found between the positive control group (treatment with metformin) and group P1. Additionally, there was a significant difference between the negative control group (no treatment) and groups P1, P2, and P3.

Discussion

The results of the study indicate that the administration of analog rice combined with moringa leaf flour (Moringa oleifera) and jackfruit seeds (Artocarpus heterophyllus) significantly reduced blood glucose levels in alloxan-induced mice, with formulation P2 (1:1 ratio) being the most effective. This effectiveness is believed to stem from the synergy between the active compounds in moringa leaves such as flavonoids (quercetin), phenols, tannins, and saponins—and the complex carbohydrates and fiber content of jackfruit seeds. Quercetin plays a crucial role through the mechanism of enhancing GLUT4 translocation to the cell membrane independently of insulin via activation of AMPK and the PI3K/Akt pathway, as demonstrated in in silico analysis showing strong affinity of quercetin for the GLUT4 receptor (−7.5 kcal/mol). Tannins and saponins further enhance the hypoglycemic effect by inhibiting glucose-digesting enzymes and stimulating pancreatic β-cell regeneration. Additionally, the high antioxidant activity of moringa leaves (IC_{50} = 1749.29 ppm) enhances protection against oxidative stress induced by alloxan. Statistically, the reduction in blood glucose levels in the treatment group was significantly proven based on ANOVA and Post Hoc Bonferroni tests. These findings not only reinforce the pharmacological evidence of local ingredients as antidiabetic agents but also indicate that functional food formulations based on moringa leaves and jackfruit seeds have the potential to be developed as preventive and therapeutic dietary interventions in the management of type 2 diabetes mellitus.

CONCLUSIONS

From the results of our research, it can be concluded that P2 rice substitute with a ratio of moringa leaf flour and jackfruit seed flour of 1:1 is proven to be more effective in lowering the blood sugar levels of alloxan-induced mice. P2 analog rice showed a significant reduction in blood sugar levels in mice compared to other rice substitute. Additionally, P2 rice substitute has a more balanced composition compared to other analog rice varieties.

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Authors' Contributions: I Gusti Ngurah Agung Adi Primantara, Komang Astri Mahadewi, and Arimbawa designing the method of this research and carried out the laboratory work. Marvel Alden Muljosaputro and Komang Dinda Bagus Putra Suryawan wrote the manuscript. I Gusti Ngurah Agung Adi Primantara analyzed the data. All authors read and approved the final version of the manuscript.

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