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EFFECT OF FERMENTATION TIME ON ANTOSIANIN CONTENT AND ANTIOXIDANT ACTIVITIES IN FERMENTED GLUTINOUS BLACK RICE EXTRACT

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ABSTRACT— Fermented Glutinous Black Rice contains compounds as antioxidants, called anthocyanin. The purpose of this study was to determine whether or not the effect of fermentation time on total anthocyanin levels and antioxidant activity. Different treatments, namely the time of fermentation, varied in the 2nd, 3rd and 4th days. Fermented Glutinous Black Rice extract was made using 1% methanol and HCl solvents. Then the samples were dried using the freeze dry method. The research was conducted to identify anthocyanins with colour reagents, test total anthocyanin levels using the differential pH method, and test antioxidant activity by looking at IC50 values. Based on the research, the highest total anthocyanin level was Fermented Glutinous Black Rice extract on the second day of 963,606 mg/100 g. The largest IC50 value is found in the extract of FermentedFermented Glutinous Black Rice on the second day at 46,313 µg/mL. Based on these results, it can be concluded that there is an effect of fermentation time on total antsoianin levels and antioxidant activity. The longer the fermentation time, the value of the total anthocyanin level and antioxidant activity decreases.

KEYWORDS: Fermented Black Glutinous Rice, extraction, anthocyanin, antioxidants.

1. INTRODUCTION

Indonesia as an agrarian country has many sources of raw materials, one of which is Fermented Glutinous Black Rice (Oryza sativa L. var glutinosa) which is quite a lot in the country of Indonesia. Glutinous rice is a plant originating from Asia which is now widespread throughout the world, including Indonesia. In some countries such as Laos and Thailand glutinous rice is used as a staple food, due to its high carbohydrate content [17]. Rice is a staple food that is consumed by many people in the world, especially in the Asian continent. Although the rice consumed is generally white, there are also rice varieties that have color pigments such as brown rice, brown rice and black rice [21]. Glutinous rice is one food that is commonly consumed as staple food or processed into flour for various cakes and snacks, besides glutinous rice is very beneficial for health which is useful for regulating the normal metabolism of fat, for growth and formation of bones and teeth. But not many people know, for the health of glutinous rice can also treat diabetes or diabetes mellitus [41]. Glutinous rice is divided into two kinds, namely white glutinous rice and black glutinous rice. This color difference depends on the pigment contained therein. Fermented Glutinous Black Rice contains anthocyanin pigment that is deep purple [36]. Fermented Glutinous Black Rice is rice that has complete nutritional value and is not inferior to other rice so that this agricultural commodity deserves to be promoted intensively as an alternative food to support a carbohydrate diversification program. This Fermented Glutinous Black Rice is high in carbohydrates, especially starch. According to Winarno (2002), amylose content of Fermented Glutinous Black Rice is 1-2%, while amylopectin is 98-99%. In food products, amylopectin is stimulating the process of blooming (puffing) in which food products derived from starch which have high amylopectin

content will be mild, porous, crisp, and crispy [24]. The bioactive component found in Fermented Glutinous Black Rice is anthocyanin. Anthocyanin is the main color component in food that can cause purple, blue, to blackish red. Anthocyanin is a polyhydroxyl or polymethoxy derivative of 2-phenyl-benzopyrylium. Anthocyanins in plants are in the form of glycosides, a component that is bound to sugar. Anthocyanin that has been released from sugar is called anthocyanidin. The types of sugars that are usually bound in anthocyanidine molecules are glucose, galactose, ramnosa, arabinose, and xylose [3]. Based on the type of group attached to the R1-R7 position, there are approximately 10 species of anthocyanidins. Anthocyanin is a typical purple pigment contained in Fermented Glutinous Black Rice and a number of studies several years back show that anthocyanin has various benefits including as an antioxidant, anti-inflammatory, anti-microbial compound, has anti-carcinogenic activity, improves vision, induces apoptosis, neuroprotective effects, affects the blood vessels and platelets thus minimizing the risk of coronary heart disease [27].

Antioxidants are free radical scavengers. The problem is when free radicals from outside enter the body. Cells in the body will be disturbed by the presence of these free radicals, resulting in radical cell mutations and abnormalities in function. Cell mutations cause cancer, nerve cell disorders, liver disorders, blood vessel disorders such as coronary heart disease, diabetes, cataracts and the causes of early aging are also triggers for other chronic diseases [15]. The advantage of the fermentation process by utilizing microbial services compared to chemical processes is that besides the process is very specific, the temperature required is relatively low and does not require metal catalysts that have pollutant properties [46]. Based on previous research, Nailufar et al. (2012) has measured the total anthocyanin and antioxidant activity of Fermented Glutinous Black Rice in Tawangsari Village, Boyolali. Total anthocyanin in 8 Fermented Glutinous Black Rice was measured at 146.47 mg / 100 g. Measured antioxidant activity of 35.73%. A decrease in total anthocyanin can occur and is influenced by several factors, including light, oxygen and temperature. The decrease in total anthocyanin will affect the antioxidant activity of Fermented Glutinous Black Rice. Packaging that can maintain antioxidant activity during storage is polypropylene (PP) plastic. Research conducted by Hou et al. (2013) identified anthocyanins as a result of isolation of black rice using HPLC and found four different anthocyanins namely cyanidin-3-11 glucoside, peonidin-3-glucoside, cyanidine-3,5-diglucoside, and cyanidine-3- routineoside. The anthocyanin content in black rice in the Sleman and Bantul regions ranged from 159.31-359.51 mg / 100g and the antioxidant activity of the DPPH method (2,2-diphenyl-1-picrylhydrazyl) was 68.968 - 85.287%. Previous research was conducted by Yannie Asrie Widanti and Nanik Suhartatik (2018), this study used a completely randomized design (CRD) with one factor, namely the difference in the types of processed products of Fermented Glutinous Black Rice. The levels of anthocyanin processed products of Fermented Glutinous Black Rice from highest to lowest are steamed sponge, oven spoon, porridge, steamed Fermented Glutinous Black Rice, Fermented Glutinous Black Rice, and rengginang. Based on the background above, there are many benefits of anthocyanin, and there is still little research on anthocyanin on Fermented Glutinous Black Rice, the purpose of the study is to know the effect of fermentation time on anthocyanin levels and antioxidant activity on Fermented Glutinous Black Rice Extract.

2. Method

This type of research is true experimental because this study aims to determine the presence or absence of the influence of anthocyanin levels and antioxidant activity in Fermented Glutinous Black Rice extracts that differ in fermentation time using the UV-VIS spectrophotometric method. The population in this study was Fermented Glutinous Black Rice obtained from the Cililin market, West Bandung. Samples are part of the population. The sample in this study was Fermented Glutinous Black Rice (processed from Fermented Glutinous Black Rice). The research site was conducted at the Integrated Laboratory of Health Polytechnic, Ministry of Health, Bandung. The implementation time is in March-May 2019. Data is obtained from primary data by measuring the absorbance and wavelength so that the amount of anthocyanin levels and antioxidant

activity in the sample can be known.

3. Result

Fermented Glutinous Black Rice extract was then tested for anthocyanin levels using the pH differential method. Data from the measurement of absorbance values at wavelengths of 510 nm and 700 nm at pH 1 and pH 4.5 are presented in table 1. The following is the result of the calculation of anthocyanin levels test on Fermented Glutinous Black Rice extract.

Table 1. Results of anthocyanin test measurements

Day	pH 1.0		pH 4,5		Absorbance Anthocyanin	Anthocyani Levels
	510 nm	700 nm	510 nm	700 nm		
2	0,414	0,160	0,053	0,030	0,231	964,361 mg/100 g
3	0.290	0.146	0,051	0,024	0,117	488,442 mg/100 g
4	0.245	0.153	0,051	0,022	0,063	263,007 mg/100 g

Analysis of antioxidant activity tests in research using the DPPH method. According to Putri, et al (2015), DPPH or 2,2-diphenyl-1-picrylhydrazil is a free radical compound that acts as an oxidizer when reacting with antioxidant compounds.

Table 2. Characteristic of Antioxidant based on IC50 values

IC_{50} Value	Characteristic of Antioxidant
< 50 ppm	Very strong
50 - 100 ppm	Strong
100 – 150 ppm	Medium
150 – 200 ppm	Weak

(Source: Molyneux, 2014)

The results of testing antioxidant activity in the study refer to table 2. The greater the IC50 value, the smaller the antioxidant properties of the sample. In this study quercetin was used as a comparison with 5 variations of concentration. The following is the result of testing the antioxidant activity of quercetin seen between concentration and% inhibition.

Table 3. Results of calculation of Antioxidant Activity test

C (ppm)	Absorbance	% Inhibition	IC_{50}
0	0,774	0	9,915
5	0,53	31,524	

10	0,298	61,499
15	0,135	82,558
20	0,071	90,827
25	0,062	91,989

From Table 3 that has been plotted, the line equation is obtained as shown in Figure 1. From the equation used to find the effective concentration of extract to reduce DPPH free radicals or IC50 values.

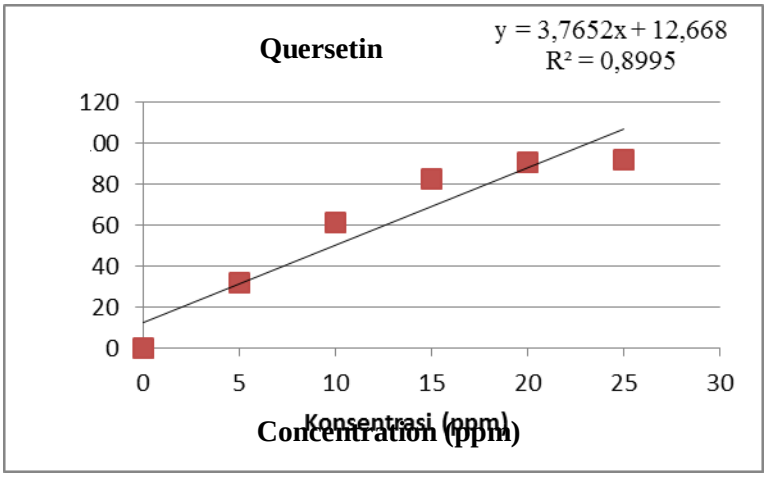

Figure 1. Graphic of the comparative antioxidant potential test (Quercetin)

IC50 value is the effective concentration of extract needed to reduce 50% of the total DPPH, so the value of 50 is substituted for the value of y. After substituting the value of 50 on the value of y, we will get the value x as the value of IC50. These results indicate an IC50 value of less than 50 µg / mL. In accordance with the parameter IC50 values in table 2, these results indicate that quercetin is a very strong antioxidant (IC50 value <50). The next step was testing the antioxidant activity of day 2 of the sample. Here are the results of the antioxidant activity of the second day of Fermented Glutinous Black Rice extract with 5 variations of concentration.

Table 4. Test results for Antioxidant Activity of Black Rice Fermented Glutinous Black Rice Extract Day 2

C (ppm)	Absorbance	%Inhibition	IC_{50}
0	0,985	0	46,313
20	0,418	57,563	
40	0,37	62,436	
60	0,368	62,640	
80	0,358	63,655	
100	0,35	64,467	

These results indicate IC50 values of less than 50 µg / mL, according to the IC50 parameter values in table 2, these results indicate that Fermented Glutinous Black Rice extract on Day 2 is a very strong antioxidant (IC50 value <50).

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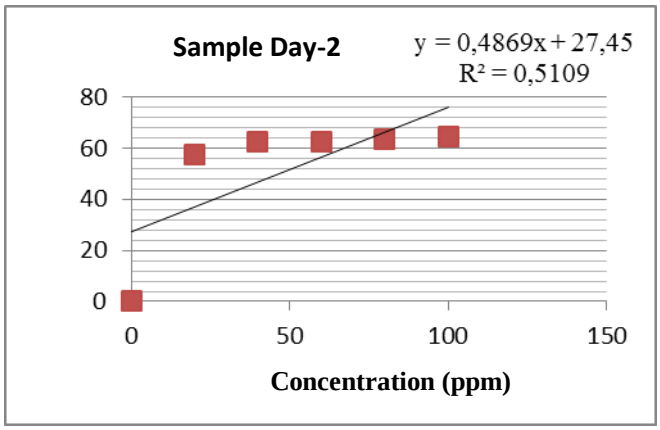

Figure 2. Graph of antioxidant potential test of Fermented Glutinous Black Rice extract

On the 3rd day sample, the same thing was done, namely the antioxidant activity test. The following are the results of testing the antioxidant activity of black rice Fermented Glutinous Black Rice extract day 3 with 5 variations of concentration.

Table 5. Test results for Antioxidant Activity of Fermented Glutinous Black Rice Extract Day 3

C (ppm)	Absorbance	%Inhibition	IC_{50}
0	0,985	0	127,558
20	0,696	29,340	
40	0,645	34,518	
60	0,64	35,025	
80	0,639	35,127	
100	0,634	35,634	

These results indicate IC50 values of more than 100 µg / mL, according to the parameters of the IC50 values in table 2, these results indicate that Fermented Glutinous Black Rice extract on Day 3 is a moderate antioxidant (IC50 value of 100-150).

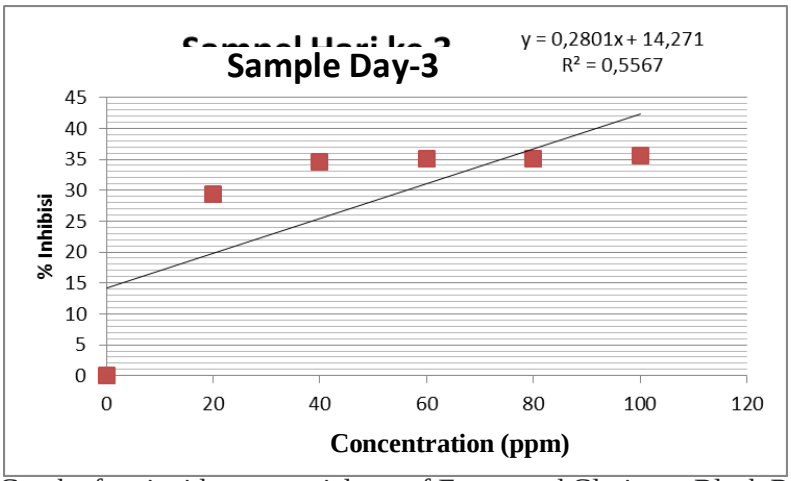

Figure 3. Graph of antioxidant potential test of Fermented Glutinous Black Rice extract

On the 4th day sample, the same thing was done, namely the antioxidant activity test. The following are the results of testing the antioxidant activity of Fermented Glutinous Black Rice extract on day 4 with 5 variations of concentration.

Table 6. Test results for the Antioxidant Activity of Black Rice Fermented Glutinous Black Rice Extract Day 4

C (ppm)	Absorbance	%Inhibition	IC_{50}
0	0,985	0	160,114
20	0,75	23,858	
40	0,734	25,482	
60	0,71	27,918	
80	0,691	29,847	
100	0,686	30,355	

These results indicate IC50 values of more than 150 µg / mL, according to the parameters of the IC50 values in table 6, these results indicate that Fermented Glutinous Black Rice extract on Day 4 is a weak antioxidant (IC50 value 150-200).

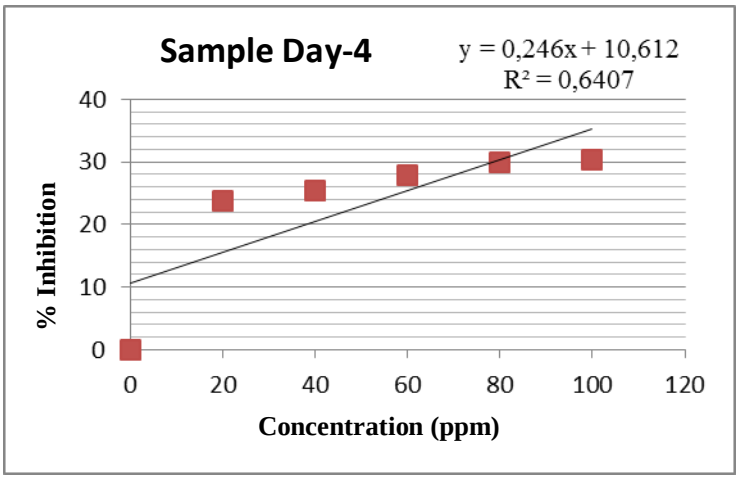

Figure 4. Graph of antioxidant potential test of Fermented Glutinous Black Rice extract

4. Discussion

This research was started by making Fermented Glutinous Black Rice which was given a different treatment, namely the difference in fermentation time. Fermentation carried out in this research is anaerobic fermentation, where the manufacturing process does not require oxygen. This research was conducted to determine whether or not the influence of fermentation time on anthocyanin levels and antioxidant activity on Fermented Glutinous Black Rice. The next step is making Fermented Glutinous Black Rice extract with maceration method. This method was chosen to avoid damage to the active substance due to heating, because the active ingredient to be extracted from Fermented Glutinous Black Rice extract is anthocyanin which functions as an antioxidant, and has heat-sensitive properties. Another reason for choosing this method is because maceration is the simplest method of extraction. The process is carried out by immersing the simplicia powder (sample) in a solvent. Robinson (1995) in Tensiska (2006) states that, extraction of flavonoid

compounds is recommended to be carried out in an acidic atmosphere, because acid serves to denaturate plant cell membranes, then dissolve anthocyanin pigments so they can get out of cells, and can prevent flavonoid oxidation. While organic acids that are often used for the extraction of pigments are hydrochloric acid, citric acid, and acetic acid. The solvents used during extraction (maceration) are methanol and hydrochloric acid. Methanol is used as a solvent because it attracts polar compounds such as flavonoids. Chloride Acid was chosen as a solvent because it is a strong acid and anthocyanins are generally more stable in acidic solutions when compared to neutral or alkaline solutions. The reason for not using water as an extraction solvent is because water is a good medium for bacterial growth, it is feared that it can damage the extract made. The reason for choosing a low temperature in the drying process is because the compound to be used is an antioxidant compound which, if given high heating, the antioxidant compound will be damaged. The anthocyanin level test and the antioxidant potential test are the main parameters of this study. Testing anthocyanin levels in this study using the pH differential method. Testing of total anthocyanin levels by the pH differential method aims to determine the total amount of anthocyanin monomers in the Fermented Glutinous Black Rice extract sample. The principle of testing total anthocyanin levels with the pH differential method is the change in anthocyanin structure due to changes in pH expressed by differences in absorbance. Measurement of sample extracts using a UV- Vis spectrophotometer was also carried out at wavelengths of 510 and 700 nm, wavelength of 510 nm was the maximum wavelength. While the purpose of measuring absorbance at a wavelength of 700 nm is to correct deposits that are still present in the sample (Giusti and Wrolstad, 2001). Anthocyanin at pH 1 forms a red to purple flavinium structure while at pH 4.5 it forms a colorless carbinol structure. Based on the results of the data obtained, the longer the fermentation time the less anthocyanin levels. This is caused by the degradation of anthocyanin caused by the hydrolysis process. According to Rein (2005) several enzymes can play a role in the process of anthocyanin degradation such as glucosidase and PPO (Polyphenol Oxidase). The glucosidase enzyme is able to stimulate the hydrolysis of the sugar bonds between the aglycone group and the glycone group. The hydrolysis causes aromatic rings to form chalcone compounds. The results of the data obtained are similar to the opinion of Giusti and Wrolstad (2001) that increasing the pH value can cause a decrease in color intensity and concentration of flavylium cation. At the same time, flavylium cation occurs hydrated to produce colorless carbinol or pseudobase. Another factor that causes anthocyanin degradation is that the analysis process on the sample is not directly carried out when the extract is ready, so that the conditions and storage period of the sample cause the anthocyanin compound to degrade. Through visual observation, the level of color density of Fermented Glutinous Black Rice can describe high levels of anthocyanin. But from the test results, although the extract showed a deep purple color, the levels of anthocyanin obtained were still relatively low due to an imperfect extraction process so that the withdrawal of anthocyanin from the Fermented Glutinous Black Rice had not been maximally attracted.

Analysis of the data obtained was done by statistical means using SPSS to determine the effect of fermentation time on anthocyanin levels contained in Fermented Glutinous Black Rice extract. From the data analysis conducted, the interpretation of the Kruskal Wallis test results in terms of homogeneity obtained sig. $P < 0.05$, so it does not meet the requirements in conducting the ANOVA test. Then proceed with the Kruskal Wallis test. P value of 0.027 ($P < 0.05$). These results indicate that there is an influence of fermentation time on total anthocyanin levels. Besides that, the antioxidant potential of Fermented Glutinous Black Rice was also carried out. The antioxidant potential test in this research is using the DPPH method. The principle of testing radical scavenging activity with the DPPH method is to measure the ability of antioxidants to capture DPPH radicals (Prior.dkk, 2005). The principle of DPPH test is based on the reaction of capturing hydrogen atoms by DPPH from antioxidant compounds. DPPH reagents act as free radicals captured by antioxidant compounds contained in the sample. In determining the antioxidant activity of Fermented Glutinous Black Rice extract, the maximum wavelength measurement is done using a standard solution. The maximum wavelength obtained is 515 nm. The wavelengths obtained are used to measure the antioxidant activity of the sample. In this study

also used quersetin as a comparison. The reason is used as a comparison because seen from the results of the antioxidant potential test, quersetin has a very strong antioxidant potential so that it can be used as a comparison in this study. When reacting Fermented Glutinous Black Rice extract with DPPH solution, the interval of a few minutes DPPH solution which was originally purple turned to fade. This shows that the DPPH solution is reduced so that it turns into DPPH-H or diphenylpicryl hydrazine. The antioxidant activity of Fermented Glutinous Black Rice extract on day 2 was obtained IC50 of 46.313%. The antioxidant activity of Fermented Glutinous Black Rice extract on the 3rd day was obtained IC50 of 127.558%. The antioxidant activity of Fermented Glutinous Black Rice extract on the 4th day was obtained IC50 of 160.114%. Based on table 4.2, the results obtained indicate that the level of antioxidant activity of Fermented Glutinous Black Rice extract on day 2 has very strong antioxidant properties, day 3 has moderate antioxidant properties, and day 4 has weak antioxidant properties. The difference in IC50 value can be caused by the amount of antioxidants contained in the extract. These results indicate that the longer the fermentation time, the less antioxidant potential of the Fermented Glutinous Black Rice extract. This occurs due to damage to antioxidants in the extract which is influenced by storage time, light, and the length of time of contact between the active substance and the solvent due to prolonged heating. In addition, antioxidants are susceptible to the heating process so that heating treatment can accelerate the oxidation of antioxidants and cause degradation of antioxidant compounds contained in natural material systems and cause a decrease in antioxidant activity in these ingredients. Damage to antioxidant compounds affects antioxidant activity. The increasing heating temperature and the duration of heating, the antioxidant activity it produces is also lower (Rifkowaty and Adha, 2016).

5. Conclusion

In addition, antioxidants are susceptible to the heating process so that heating treatment can accelerate the oxidation of antioxidants and cause degradation of antioxidant compounds contained in natural material systems and cause a decrease in antioxidant activity in these ingredients. Damage to antioxidant compounds Aff antioxidant activity. The increasing heating temperature and the duration of heating, the antioxidant activity it produces is also lower (Rifkowaty and Adha, 2016). The duration of fermentation affects the total levels of anthocyanin and antioxidant potential. The longer the fermentation time, the value of total anthocyanin levels of Fermented Glutinous Black Rice extract decreases and the value of antioxidant potential possessed by Fermented Glutinous Black Rice extract is increasing, meaning that its antioxidant potential decreases.

6. Suggestion

Further research needs to be done regarding the identification of the type of anthocyanin contained in Fermented Glutinous Black Rice extract using HPLC. For Fermented Glutinous Black Rice traders, it is better to make a Fermented Glutinous Black Rice with fermentation time of 2 to 3 days, because at the time of fermentation the total levels of anthocyanin and its antioxidant potential are high.

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