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by Elvi Trinovani

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THE EFFECT OF DIFFERENCES YEAST'S CONCENTRATION ON TOTAL ANTHOCYANIN CONTENT, AND ANTIOXIDANT ACTIVITIES OF BLACK TAPE EXTRACT *(ORYZA SATIVA L VAR FORMA GLUTINOSA)*

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ABSTRACT

Anthocyanin is a sub-type organic substance from the flavonoid, and is one example of natural-colored pigments. This substance can be found in food items such as Fermented Black Glutinous Rice. Aside from anthocyanin, previously studied Fermented Black Glutinous Rice samples also possess antioxidant activity that can be developed to prevent hyperlipidemia. However, the fermenting process on the black rice is known to affect the anthocyanin substance and the antioxidant activity contained within the black rice. This study is conducted to find out the specific effects caused by the yeast and its concentrations on the fermenting process. The results show that the yeast concentrations do affect the anthocyanin level and the antioxidant activity in the black rice. The anthocyanin level, after subjected to some pH differential tests, plummeted as the level of yeast concentrations went up. The highest anthocyanin level was obtained in the sample with 0.5% yeast concentration which was equal to 566,0226 mg / 100g. Likewise, the results of examination of antioxidant activity have decreased with increasing yeast concentration. The highest antioxidant activity was obtained at 0.5% yeast concentration. This is showed by the IC50 value of 81.9834 ppm which means the sample has strong antioxidant activity.

Keyword : Fermented Black Glutinous Rice, Anthocyanin, Antioxidant Activity.

Introduction

Anthocyanins are a sub-type of organic compounds from the flavonoid family. Some of the most common anthocyanin compounds are pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delfinidin (Karnajanawipagul et al, 2010). Anthocyanins work as secondary antioxidants as is the case with β-carotene, which breaks down the oxidacidipid peroxide chain. Antioxidants are free radical scavengers or antidotes (Lucioli, 2012).

The source of anthocyanin and fiber besides fruits and vegetables is rice (*Oryza sativa*) which is rich in anthocyanin such as black Glutinous Black Rice , black rice and brown rice (Itani, 2004). Fermented Glutinous Black Rice (*Oryza sativa glutinosa*) as a raw material for Fermented Glutinous Black Rice tape is a very potential commodity as a source of carbohydrates, antioxidants, bioactive compounds and fibers that are important for health (Rooney, 2000). One of the foods in Indonesia made from Glutinous Black Rice is Fermented Glutinous Black Rice containing ferocyanin, phenols and antioxidant activity. Glutinous tape has anthocyanin content of 257 ppm or equivalent to 25.7 mg / 100 grams. (Elisa, 2013).

Glutinous Black Rice tape is a traditional Indonesian food made from Glutinous Black Rice which is processed by fermentation. In the Glutinous Black Rice fermentation process the preparation (washing, cooking and cooling) stages, workmanship, and fermentation (heating, inoculation with yeast, and incubation) are important influential stages to produce good quality Glutinous Black Rice tape (Hidayat et al, 2006).

The process of making Fermented Glutinous Black Rice is done by washing Fermented Glutinous Black Rice to clean dirt and contamination. Furthermore, black glutinous rice soaked for several hours. Soaking aims to produce Fermented Glutinous Black Rice that is not hard and shorten the steaming time. Then black rice that has been soaked, boiled half done and then steamed (Fauziyah, 2015).

The steaming process will cause the starch to gelatinize and will break into amylose and amylopectin. Gelatinized starch is used as a growth medium for microbes present in yeast. Before the fermentation of Glutinous Black Rice is carried out, it is cooled first until the temperature approaches room temperature so that the microbes present in the yeast can work optimally. Add yeast with a concentration of 0.1% -0.5% because at that concentration will produce a tape with a sweet taste, sour and distinctive aroma tape. The yeast used for fermentation is Sacharomyces *cereviseae* (Fauziyah, 2015).

In addition to influencing the taste of food, the provision of yeast in the fermentation process can affect the ingredients in food. Among them the fermentation method which gives a very significant effect on anthocyanin levels of sweet potato flour during the fermentation process (Juliana et al, 2017). It is also as stated by Medina (2018) that yeast has been shown to interact with anthocyanins for example is interactions with yeast cell wall material. So that it allows a decrease in anthocyanin levels during the Fermented Glutinous Black Rice fermentation process. The use of varied yeast in each yeast-producing region can also be one of the factors that influence the fermentation process.

Cililin area, West Bandung Regency is one of the regions producing Fermented Glutinous Black Rice. The use of yeast during Fermented Glutinous Black Rice fermentation in the Cililin area still uses concentrations in accordance with a pre-existing recipe of 0.1%. At the concentration of the yeast indeed obtained tape with optimum conditions. However, there is no known concentration of yeast that can provide maximum levels of anthocyanin and antioxidant activity on Fermented Glutinous Black Rice.

Based on the background that has been explained, it is known that there are many benefits of anthocyanin, but there are several things that can affect anthocyanin levels when making Fermented Glutinous Black Rice tape and the need to illustrate the effect of yeast concentration on anthocyanin levels and antioxidant activity of Fermented Glutinous Black Rice in order to obtain optimum yeast concentration. So the authors are very interested in conducting a study entitled "Effect of Differences in Yeast Concentration on Total Anthocyanin Levels, And Antioxidant Activity of Fermented Glutinous Black Rice Extract (*Oryza sativa L var forma glutinosa*).

Method

This type of research is a descriptive study to determine the effect of different levels of yeast given on total anthocyanin levels and antioxidant activity on Fermented Glutinous Black Rice. This study uses Fermented Glutinous Black Rice obtained from Cililin in West Bandung Regency, West Java.

The study was conducted at the Bandung Health Polytechnic Laboratory, Department of Pharmacy. The time of the research was carried out in March 2019. Materials used included test materials and chemicals. Test materials used are Black Glutinous Rice and Chemicals used include Aquabides, KCl 0.2 N solution; HCl 0.2 N solution; potassium acetate, ascorbic acid, methanol pa merck brand, DPPH (*1,1-diphenyl-2-picrylhydrazyl*), [(CH3COO) 2Cu.H2O], [(CH3COO) 2Mg.4H2O], ethanol pa brand merck, filter paper whatman no. 42, aluminum foil and tissue roll. The tools used include UV-Vis spectrophotometers, glass cuvettes, pH meters, analytical scales, water baths, centrifugers, mercury thermometers, glassware, stopwatches, filters and parchment paper.

The workings of this study began with the preparation of test material in the form of Fermented Glutinous Black Rice. Then the fermentation process is carried out, the results of which will be tested, namely, identification of anthocyanin pigment compounds, analysis of maximum wavelength, determination of anthocyanin levels and antioxidant activity test using DPPH method

Result

The plants used in this research are Fermented Glutinous Black Rice (Oryza sativa L.) Poaceae tribe which has been fermented into Fermented Glutinous Black Rice taken from the Poltekkes Bandung Village of Cililin Village, West Bandung Regency. This research was conducted using 5 groups of Fermented Glutinous Black Rice samples with several different concentrations of yeast.

Before testing the sample, extraction is done first with the aim of being able to take anthocyanin compounds that are deposited in the sample. In this research, extraction was done

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using maceration method which was then continued with freezedry. The sample extraction results obtained are in the table.

Table 1 Yield of Fermented Glutinous Black Rice Extract with 0.05% Yeast Concentration

Solvent	Methanol + HCl 1%
Sample Weight	250 g
Extract Weight	20,8494 g
Yield Extract	8,34 %

Table 2 Yield of Fermented Glutinous Black Rice Extract with 0.075% Yeast Concentration

Solvent	Methanol + HCl 1%
Sample Weight	250 g
Extract Weight	23,7579 g
Yield Extract	9,50 %

Table 3 Yield of Fermented Glutinous Black Rice Extract with 0.1% Yeast Concentration

Solvent	Methanol + HCl 1%
Sample Weight	250 g
Extract Weight	21,1781 g
Yield Extract	8,54 %

Table 4 Yield of Fermented Glutinous Black Rice Extract with 0.125% Yeast Concentration

Solvent	Methanol + HCl 1%
Sample Weight	250 g
Extract Weight	22,3693 g
Yield Extract	8,95 %

Table 5 Yield of Fermented Glutinous Black Rice Extract with 0.15% Yeast Concentration

Solvent	Methanol + HCl 1%
Sample Weight	250 g
Extract Weight	19,6600 g
Yield Extract	7,86 %

Anthocyanin identification can be done by doing color reagents by reacting the extract with HCl and NaOH. The results of identifying the color of Fermented Glutinous Black Rice extract with color reagents are in table 6.

Table 6 Results of Anthocyanin Identification with Color Reagents

Reactor	Color	Identification results
HCl	Purplish red	+
NaOH	Color is fading	+

Determination of anthocyanin levels is done by the pH differential method. Determination of anthocyanin levels is done by using a pH difference that is pH 1.0 and pH 4.5. This is done because at pH 1.0 anthocyanin forms a red flavylium / oxonium ketion. Meanwhile, at a pH of 4.5 anthocyanins form a colorless carbinol compound. Thus, the determination of levels can be done by making aliquots of anthocyanin solutions in pH 1.0 and pH 4.5 to then be measured for absorbance.

The sample used was Fermented Glutinous Black Rice extract with some concentration of yeast. Samples that have been dissolved with different pH buffer solutions are then inserted in a spectrophotometer to determine the absorbance of each solution. Following is the absorbance results table for each Fermented Glutinous Black Rice extract solution with a yeast concentration of 0.05%.

Table 7 Anthocyanin levels of Fermented Glutinous Black Rice Extract 0.05% Yeast Concentration

	Replication	1	2	3
pH 4,5	515 nm	0,465	0,477	0,484
	700 nm	0,200	0,219	0,204
pH 1	513 nm	2,356	2,263	2,216
	700 nm	0,644	0,600	0,604
Anthocyanin Absorption		1,447	1,405	1,332
Anthocyanin levels (mg/100g)		604,0822	586,5483	556,0729
Average of Anthocyanin levels			582,2344 mg/100g	

Absorbance samples that have been measured using a spectrophotometer in wavelengths of 510 nm and 700 nm can be calculated for anthocyanin levels by determining the absorbance of the sample first. The absorbance of each solution is calculated based on the equation found in the example calculation. The total anthocyanin monomeric from the dried extract of Fermented Glutinous Black Rice was calculated as cyaniding-3glucoside.

The results of anthocyanin levels from methanol extract and HCl Fermented Glutinous Black Rice tape with a concentration of 0.75% can be seen in table 8

Table 8 Anthocyanin Levels of Fermented Glutinous Black Rice Extract 0.075% Yeast Concentration

	Replication	1	2	3
pH 4,5	515 nm	0,328	0,329	0,331
	700 nm	0,177	0,173	0,181
pH 1,0	513 nm	1,919	1,987	1,922
	700 nm	0,533	0,523	0,525
Anthocyanin Absorption		1,303	1,308	1,247
Anthocyanin levels (mg/100g)		543,9662	546,0535	520,5877
Average of Anthocyanin levels			536,8691mg/100g	

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From the table calculation of anthocyanin levels above, obtained anthocyanin levels in Fermented Glutinous Black Rice extract with a yeast concentration of 0.5% amounted to 536.8691 mg / 100g.

The results of anthocyanin levels from methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 1% can be seen in table 9

Table 9 Anthocyanin Levels of Fermented Glutinous Black Rice Extract 0.1% Yeast Concentration

	Replication	1	2	3
pH 4,5	515 nm	0,283	0,268	0,231
	700 nm	0,106	0,100	0,094
pH 1,0	513 nm	1,364	1,354	1,295
	700 nm	0,278	0,304	0,283
Anthocyanin Absorption		0,909	0,882	0,875
Anthocyanin levels (mg/100g)		379,4822	368,2104	365,2881
Average of Anthocyanin levels			370,9936 mg/100g	

From the calculation table of anthocyanin levels above, obtained anthocyanin levels in Fermented Glutinous Black Rice extract with 1% yeast concentration is 370.9936 mg / 100g.

The results of anthocyanin test results from methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 1.25% can be seen in table 10.

Table 10 Anthocyanin levels of Fermented Glutinous Black Rice Extract Yeast Concentration 0.125%

	Replication	1	2	3
pH 4,5	515 nm	0,127	0,111	0,113
	700 nm	0,092	0,089	0,089
pH 1,0	513 nm	0,663	0,666	0,692
	700 nm	0,155	0,166	0,189
Anthocyanin Absorption		0,473	0,478	0,479
Anthocyanin levels (mg/100g)		197,4643	199,5517	199,9691
Average of Anthocyanin levels			198,9950 (mg/100g)	

From the calculation of anthocyanin levels above, obtained anthocyanin levels in Fermented Glutinous Black Rice extract with a yeast concentration of 1.25% amounted to 198.995 mg / 100g.

The results of anthocyanin test results from methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.15% can be seen in table 11.

From the calculation table above, obtained anthocyanin levels in Fermented Glutinous Black Rice extract with a yeast concentration of 0.15% amounted to 189.1149 mg / 100g.

Table 11 Anthocyanin levels of Fermented Glutinous Black Rice Extract Yeast Concentration of 0.15%

	Replication	1	2	3
pH 1,0	515 nm	0,104	0,106	0,105
	700 nm	0,083	0,087	0,088
pH 4,5	513 nm	0,544	0,540	0,543
	700 nm	0,064	0,071	0,076
Anthocyanin Absorption		0,459	0,450	0,450
Anthocyanin levels (mg/100g)		191,6197	187,8625	187,8625
Average of Anthocyanin levels			189,1149 (mg/100g)	

From all samples of Fermented Glutinous Black Rice extract that have been tested, there are differences in anthocyanin levels. The results of the calculation of anthocyanin levels from Fermented Glutinous Black Rice extract with various yeast concentrations can be seen in table 12.

Table 12 Results of Anthocyanin Analysis on Fermented Glutinous Black Rice Extract

Sample	pH 4.5		pH 1.0		Anthocyanin Absorption	Anthocyanin levels (mg/100g)
	515 nm	700 nm	515 nm	700 nm		
Sample [1]	0,475	0,208	2,240	0,616	1,356	566,0226
Sample [2]	0,329	0,177	1,955	0,527	1,275	532,3465
Sample [3]	0,261	0,100	1,338	0,288	0,889	370,9936
Sample [4]	0,117	0,090	0,674	0,170	0,477	198,995
Sample [5]	0,105	0,086	0,542	0,070	0,453	189,1149

Note :

[1] Samples with 0.05% yeast concentration

[2] Samples with 0.075% yeast concentration

[3] Samples with 0.1% yeast concentration

[4] Samples with 0.125% yeast concentration

[5] Samples with 0.15% yeast concentration

One method that is often used to determine the antioxidant activity of Fermented Glutinous Black Rice is the DPPH method. In measuring antioxidant activity, five concentrations of using yeast during fermentation were used to determine the effect of yeast during fermentation on the antioxidant activity of Fermented Glutinous Black Rice. The antioxidant activity of black crab tape with some yeast concentration during fermentation is indicated by the IC50 value.

The results of antioxidant activity tests of methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.05% can be seen in table 13

The data in table 13 is the absorbance data of Fermented Glutinous Black Rice extract with a yeast concentration of 0.05% obtained after measurements using a UV-Vis spectrophotometer. Obtained a decrease in absorbance along with an increase in the concentration of the sample solution. Absorbance data is then calculated% inhibition or% of antioxidant activity with a predetermined formula. Then the results are used to calculate

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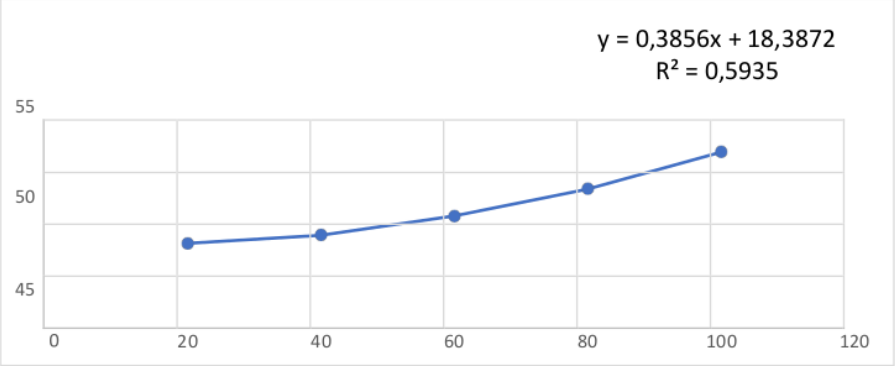
Table 13 Antioxidant Activity of Fermented Glutinous Black Rice Extract 0.05% Yeast Concentration

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,985	0	y = 0,3856x + 18,3872 R^2 = 0,5935	81,983
20	0,574	41,72589		
40	0,5663	42,50761		
60	0,5483	44,33503		
80	0,5227	46,93401		
100	0,4877	50,48731		

C50 which is the value to measure antioxidant activity with the linear equation y = ax + b. IC50 equation that has been obtained can be illustrated by graph 1.



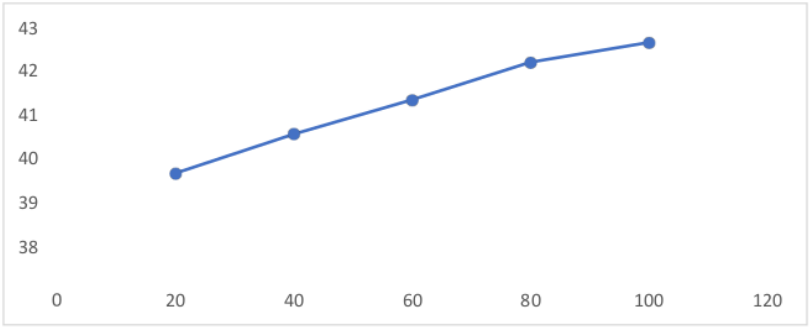
Graph 1 Antioxidant Activity of Fermented Glutinous Black Rice Extract 0.05% Yeast Concentration

The results of antioxidant activity tests of methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.075% can be seen in table 14. Based on table 14 IC50 values or the concentration of methanol extract and HCl Fermented Glutinous Black Rice tape with a yeast concentration of 0.075% inhibiting 50% of DPPH free radicals at 100.531 ppm.

Table 14 Antioxidant Activity of Fermented Glutinous Black Rice Extract 0.075% Yeast Concentration

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,985	0	y = 0,3207x + 17,7597 R^2 = 0,5208	100,531
20	0,6083	38,24365		
40	0,5957	39,52284		
60	0,5847	40,63959		
80	0,5727	41,85787		
100	0,5663	42,50761		

IC50 equation that has been obtained can be illustrated by graph 2.



Graph 2 Antioxidant Activity of Fermented Glutinous Black Rice Extract 0.075% Yeast Concentration

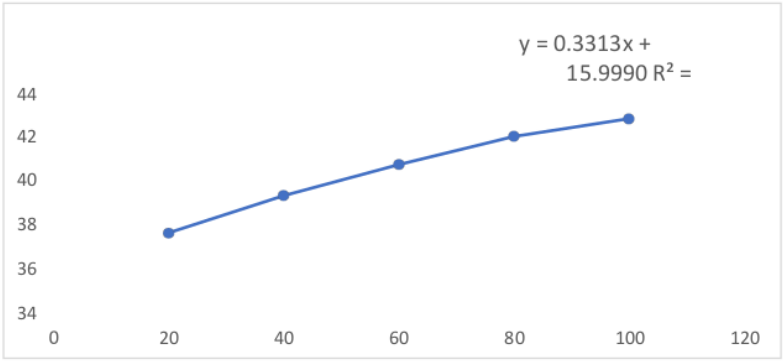
The results of antioxidant activity tests of methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.1% can be seen in table 15.

Table 15 Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration 0.1%

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,985	0	y = 0,3313x + 15,9990 R^2 = 0,58806	102,629
20	0,6397	35,05584		
40	0,6163	37,43147		
60	0,597	39,39086		
80	0,5793	41,18782		
100	0,5683	42,30457		

Based on table 15, the IC50 value or the concentration of methanol extract and HCl Fermented Glutinous Black Rice tape with a yeast concentration of 0.1% inhibiting 50% of DPPH free radicals is 102.662 ppm.

IC50 equation that has been obtained can be illustrated by graph 3.



Graph 3 Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration 0.1%

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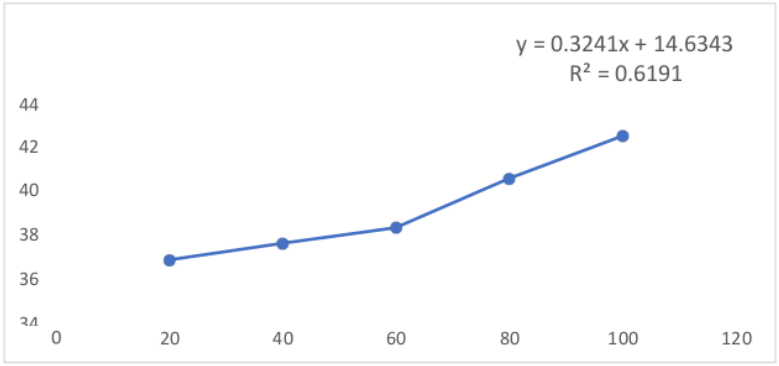
The results of antioxidant activity tests of methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.125% can be seen in table 16.

Table 16. Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration 0.125%

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,985	0	y = 0,3241x + 14,6343 R^2 = 0,6191	109,12
20	0,654	33,6041		
40	0,643	34,7208		
60	0,6327	35,7665		
80	0,6003	39,0558		
100	0,5723	41,8985		

Based on table 4.15, the IC50 value or the concentration of methanol extract and HCl Fermented Glutinous Black Rice tape with a yeast concentration of 0.125% inhibits 50% of DPPH free radicals at 109.12 ppm.

IC50 equation that has been obtained can be illustrated by graph 4.



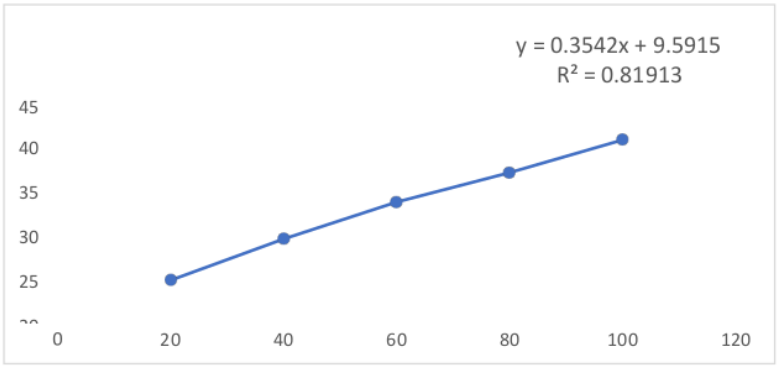
Graph 4. Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration 0.125%

The results of antioxidant activity tests of methanol extract and HCl Fermented Glutinous Black Rice with a concentration of 0.15% can be seen in table 17

Table 17 Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration of 0.15%

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,985	0	y = 0,3542x + 9,5915	114,084
20	0,75	23,858	R^2 = 0,81913	
40	0,7007	28,863		
60	0,6567	33,330		
80	0,6217	36,883		
100	0,5823	40,883		

Based on table 17, the IC50 value or the concentration of methanol extract and HCl Fermented Glutinous Black Rice tape with a yeast concentration of 0.15% inhibits 50% of DPPH free radicals of 114.08192 ppm. IC50 equation that has been obtained can be illustrated by graph 5.



Graph 5 Antioxidant Activity of Fermented Glutinous Black Rice Extract Yeast Concentration of 0.15%

From all the Fermented Glutinous Black Rice extract samples that were tested there were differences in IC50 values. IC50 calculation results from Fermented Glutinous Black Rice extract with various yeast concentrations can be seen in table 18.

Table 18 Results of Antioxidant Activity Analysis of Fermented Glutinous Black Rice Extract

Sample	IC 50 (ppm)
1	81.9834
2	100.531
3	102.629
4	109.12
5	114,084

Note :

[1] Samples with 0.05% yeast concentration

[2] Samples with 0.075% yeast concentration

[3] Samples with 0.1% yeast concentration

[4] Samples with 0.125% yeast concentration

[5] Samples with 0.15% yeast concentration

Based on table 18 IC50 values or the concentration of methanol extract and HCl Fermented Glutinous Black Rice tape with some yeast concentrations inhibiting 50% of DPPH free radicals increased with an increase in yeast concentration. IC7 reduction that has been obtained can be illustrated by graph 6.

The comparison used in this study was quercetin which was also tested by DPPH by measuring the absorbance of the comparator after adding DPPH reagents first. Then, the comparative absorbance results are used to calculate the 50% inhibitory concentration of free radicals / IC50. The results of antioxidant activity tests from quercetin comparator can be seen in table 19

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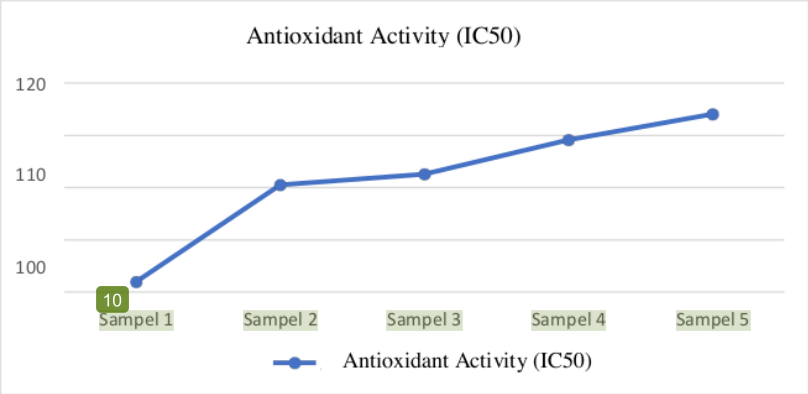
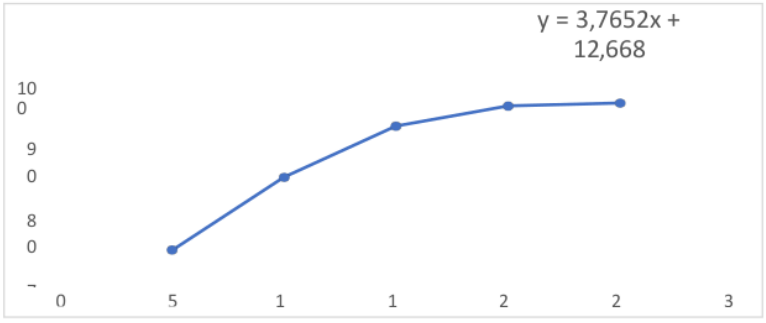


Gafik 6 Comparison of Antioxidant Activity for each Concentration

Table 19. Antioxidant Activity of Quercetin

Concentration (ppm)	Absorbance	% Inhibition	Equation (y=bx+c)	IC50 (ppm)
0	0,774	0	y = 3,7652x + 12,668	9,915
5	0,53	31,5245	R^2 = 0,8995	
10	0,298	61,4987		
15	0,135	82,5581		
20	0,071	90,8269		
25	0,062	91,9897		

Based on table 19, the IC50 value or quercetin comparative concentration inhibiting 50% of DPPH free radicals is 9,915 ppm. IC50 equation that has been obtained can be illustrated by graph 7.



Graph 7 Quercetin Comparative Antioxidant Activity

Discussion

This research was carried out on Fermented Glutinous Black Rice with five concentrations of yeast fermentation to determine its effect on anthocyanin levels and antioxidant activity. Before testing the sample, extraction is done first. Extraction is the first step in determining the characterization of pigments so that coarse extracts are obtained. In fruits or vegetables, anthocyanin pigments are generally found in cells located near the surface. Anthocyanin contained in these tissues can be obtained by extraction using certain solvents. One theory says that the extracting agent can cause denaturation of the cell membrane so that the pigment contained in the membrane can be extracted (Francis, 1982).

The effectiveness of the extraction process is inseparable from the ability of the extracting material to dissolve the extracted component. Dissolution event of a substance occurs because of the interaction between the solvent and the dissolved material and can be divided into three stages, namely, the stage of breaking bonds between molecules of solutes that require energy, the stage of breaking bonds between solvent molecules that require energy, and finally the stage of formation a bond between a solute molecule and an energy-solvent molecule. If the energy produced is greater than the energy required, the dissolution process will occur. Polar compounds can only dissolve in polar solvents, as well as non-polar compounds which can only be dissolved in non-polar solvents as well (Nur et al., 1981).

This maceration process is carried out twice 24 hours to produce a purple filtrate. This is done to optimize the extraction process so that the anthocyanin pigment contained in the Fermented Glutinous Black Rice can be extracted entirely. Stirring with the stirer is done to increase the effectiveness of the extraction process. The resulting filtrate is then concentrated by the freezedry method. The use of low temperatures aims to avoid the degradation and hydrolysis of anthocyanin pigments (Timberlake and Bridle, 1983). The extract powder obtained is then put in a dark bottle and stored in the freezer. Storage in the freezer aims to maintain the stability of anthocyanin which is very easily degraded.

This analysis was carried out using a UVVis spectrophotometer at two wavelengths, 515 and 700 nm. The wavelength of 515 nm is the maximum wavelength of anthocyanin Fermented Glutinous Black Rice. This result is obtained by dissolving the Fermented Glutinous Black Rice extract in buffer pH 1 which is then measured its maximum wavelength.

The average results of anthocyanin levels from Fermented Glutinous Black Rice extract with 0.05% yeast concentration was 566.0226 mg / 100g, for Fermented Glutinous Black Rice extract with 0.075% yeast concentration was 532.33465 mg / 100g, for Fermented Glutinous Black Rice extract with 0.1% yeast concentration is 370.9936 mg / 100g, for Fermented Glutinous Black Rice extract with 0.125% yeast concentration is 198.995 mg / 100g, for Fermented Glutinous Black Rice extract with 0.15% yeast concentration is 189.111149 mg / 100g . The analysis showed that the higher the yeast concentration in fermented Fermented Glutinous Black Rice, the less anthocyanin levels contained in the sample.

The effect of decreased levels of anthocyanin occurs due to the absorption of anthocyanin by the yeast cell wall. This is in accordance with Morata's research, 2016 concerning the Effect of Yeast on Khamr Color states that the yeast cell wall can adsorb anthocyanin pigments. Although the adsorption is only in a small color, it is reported that S. Serevicae chain can absorb anthocyanin in the range of 1.6 - 5.8% of anthocyanin levels.

Besides that, the level of anthocyanin contained in Fermented Glutinous Black Rice which has undergone a fermentation process can decrease due to the heating stage in making Fermented Glutinous Black Rice. One factor that occurs during cooking is the development of starch grains from glutinous rice and rice which have an impact on the physical damage to the pericarp layer. Cooking causes the development of starch grains which have an impact on physical damage to the pericarp layer. The composition of starch which almost all consists of amylopectin causes Glutinous Black Rice does not expand in cooking (Mambrasar et al., 2010)

In addition, statistical testing was carried out to determine the effect of adding yeast to anthocyanin levels. Obtained normality values so that the test is done with the Kruskall Wallis

and Mann Whitney test. From the test results obtained that at the concentration of yeast 0.05%, 0.075%, 0.1% and 0.125% p value >0.5 so that there are no significant differences. Meanwhile, at a concentration of 0.15% the value of p <0.5, which means that there is a meaningful difference, or the concentration has a difference with other concentrations.

DPPH trapping is often carried out for testing antioxidants from cysteine, glutathione, ascorbic acid, tocopherols, and the aromatic components of polyhydroxyl, olive oil, fruits, fruit juices and wine fermentation. The advantage of this method is that DPPH will act with the sample as a whole within a certain time and DPPH can react slowly even with weak antioxidants (Prakash, 2001). Antioxidant activity is related to rice type. Rice contains compounds such as tocopherol, orizanol and polyphenols. Polyphenols such as vanilla acid, quercetin, and other phenolics are known to be potential antioxidants. Low molecular weight polysaccharides, proteins or peptides also affect antioxidants (Palaniveloo and Vairappan, 2013). Fermented Glutinous Black Rice and black rice are known to contain anthocyanin compounds which give a black color to the pericarp layer. In addition, the results obtained increase in the value of IC50 with an increase in the concentration of yeast in the sample. Increased IC50 illustrates a decrease in antioxidant activity. Because, the smaller the IC50 value, the higher the free radical scavenging activity (Molyneux, 2004). This is like what happened in the study (Prabhu, 2014) the total antioxidant activity decreases in the presence of fermentation, because without the addition of oxygen, plant sterols quickly disrupt the yeast membrane's properties by becoming dominant dominant, causing slow fermentation. So during the 48th hour of fermentation many sterols are used by yeast so there is a decrease in total antioxidant activity.

Conclusion

Terdapat pengaruh konsentrasi ragi berupa penurunan hasil kadar antosianin dan aktivitas antioksidan pada setiap kenaikan konsentrasi ragi pada sampel.Didapatkan nilai kadar antosianin tertinggi sebesar 582.2344 mg/100g pada konsentrasi ragi 0,05% dan nilai aktivitas antioksidan tertinggi yang ditunjukkan dengan nilai IC50 terendah sebesar 81,983 ppm pada konsentrasi ragi 0,05%.

Sugesst

Further research needs to be done to identify the type and quality of anthocyanin contained in Fermented Glutinous Black Rice more precisely such as using HPLC, so that the type and quality of anthocyanin can be known appropriately and it is necessary to improve the stability of the anthocyanin pigment contained in the Fermented Glutinous Black Rice so that it can be utilized as a source of natural dyes and herbal treatments.

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